STRUCTURAL STUDIES ON POLYSACCHARIDES.

A thesis submitted for the degree

of Doctor of Science

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by

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FOREWORD.

This thesis consists of reprints of published work carried out wholly or in part under the writer's supervision, together with a brief introduction in which the general field of investigation is outlined. In four of the publications * (10, 17, 25, 35) a considerable part of the experimental work was performed by the writer and in the other papers the experimental work, carried out by research students or by post-doctoral research workers, was directly supervised by the writer. A number of the publications (2, 3, 5, 9, 12, 15, 16, 19, 34 and 35) describe work carried out in collaboration with Professor E. L. Hirst, C.B.E., F.R.S., as part of the general programme of research in the Department of Chemistry of the University of Edinburgh. Three papers (13, 22, 31) describe the results of investigations undertaken in collaboration with senior workers in other laboratories; in these cases, the detailed chemical studies were carried out under the writer's supervision. One review article (21) was written in collaboration with a colleague. The one paper (38), which is submitted in manuscript form, has been accepted for publication in the Journal of the Chemical Society and will appear in April, 1958.

* Figures in parenthesis refer to the list of papers in the thesis.

Publications

1. The Isolation of Sucrose from the Partial Hydrolysis of the Fructosan from *Lolium perenne*. By G. O. Aspinall and R. G. J. Telfer. *Chemistry & Industry*, 1952, 1244-1245.

2. Studies on Fructosans. Part IV. A Fructosan from *Dactylis glomerata*. By G. O. Aspinall, E. L. Hirst, E. G. V. Percival and R. G. J. Telfer. *J. Chem. Soc.*, 1953, 337-342.

3. The Hemicelluloses of Esparto Grass (*Stipa tenacissima*, L.). The Arabinose-rich Fraction. By G. O. Aspinall, E. L. Hirst, R. W. Moody and E. G. V. Percival. *J. Chem. Soc.*, 1953, 1631-1634.

4. The Occurrence of Glucose Residues in Inulin. By G. O. Aspinall and R. G. J. Telfer. *Chemistry & Industry*, 1953, 490.

5. The Mannans of Ivory Nut (*Phytelephas macrocarpa*). Part I. The Methylation of Mannan A and Mannan B. By G. O. Aspinall, E. L. Hirst, E. G. V. Percival and I. R. Williamson. *J. Chem. Soc.*, 1953, 3184-3188.

6. The Chemistry and Chemical Degradation of Cellulose. By G. O. Aspinall. *Biochem. Soc. Symposium*, 1953, No. 11, 42-48.

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8. The Constitution of a Wheat-straw Xylan. By G. O.

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11. The Methyl Ethers of Hexuronic Acids. By G. O. Aspinall. Advances in Carbohydrate Chem., 1954, 9, 131-148.

12. The Alkali-soluble Polysaccharides of the Lichen Cladonia alpestris (Reindeer Moss). By G. O. Aspinall, E. L. Hirst and (Mrs.) Margaret Warburton. J. Chem. Soc., 1955, 651-655.

13. The Extracellular Polysaccharide of Aerobacter aerogenes A3 (S1) (Klebsiella Type 54). By J. F. Wilkinson, W. F. Dudman and G. O. Aspinall. Biochem. J., 1955, 59, 446-451.

14. Studies on Fructosans. Part VI. The Degradation of Fructosans in Aqueous Solution. By G. O. Aspinall and R. G. J. Telfer. J. Chem. Soc., 1955, 1106-1110.

15. Gum Ghatti (Indian Gum). The Composition of the Gum and the Structure of Two Aldobiouronic Acids derived from it. By G. O. Aspinall, E. L. Hirst and A. Wickstrom. J. Chem. Soc., 1955, 1160-1165.

16. The Constitution of a Modified Starch from Malted Barley. By G. O. Aspinall, E. L. Hirst and W. McArthur.

J. Chem. Soc., 1955, 3075-3081.

17. The 1:4-Lactone of (±) 2:4-Dihydroxy-2-hydroxymethyl-butanoic Acid, a New Saccharinolactone. By G. O. Aspinall, (Miss) Mary E. Carter and M. Los. Chemistry & Industry, 1955, 1553-1554.

18. Studies on Degraded Esparto Cellulose. By G. O. Aspinall and W. B. Fordyce. J. Chem. Soc., 1956, 683-687.

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24. The Constitution of a Wheat-straw Hemicellulose. By G. O. Aspinall and Eric G. Meek. J. Chem. Soc., 1956, 3830-3834.

25. The Degradation of Xylobiose and Xylotriose by Alkali. By G. O. Aspinall, (Miss) Mary E. Carter and (in part) M. Los. J. Chem. Soc., 1956, 4807-4810.

26. Selective Esterification of Equatorial Hydroxyl Groups in the Synthesis of Some Methyl Ethers of D-Mannose. By G. O. Aspinall and G. Zweifel. J. Chem. Soc., 1957, 2271-2278.

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28. A Spectrophotometric Method for the Determination of Periodate Consumed during the Oxidation of Carbohydrates. By G. O. Aspinall and R. J. Ferrier. Chemistry & Industry, 1957, 1216.

29. The Structure of Callose from the Grape Vine. By G. O. Aspinall and G. Kessler. Chemistry & Industry, 1957, 1296.

30. The Constitution of Barley Husk Hemicellulose. By G. O. Aspinall and R. J. Ferrier. J. Chem. Soc., 1957, 4188-4194.

31. The Glucomannans from Sitka Spruce (Picea sitchensis). By G. O. Aspinall, R. A. Laidlaw and R. B. Rashbrook. J. Chem. Soc., 1957, 4444-4448.

32. Cereal Gums. Part II. The Constitution of an Araboxylan from Rye Flour. By G. O. Aspinall and R. J. Sturgeon. J. Chem. Soc., 1957, 4469-4471.

33. The Mannans of Ivory Nut (*Phytelephas macrocarpa*). Part II. The Partial Acid Hydrolysis of Mannans A and B. By G. O. Aspinall, R. B. Rashbrook and (in part) G. Kessler. *J. Chem. Soc.*, 1958, 215-221.

34. Gum Ghatti (Indian Gum). Part II. The Hydrolysis Products from the Methylated Degraded Gum and the Methylated Gum. By G. O. Aspinall, (Mrs.) Barbara J. Auret and E. L. Hirst, *J. Chem. Soc.*, 1958, 221-230.

35. The Constitution of Larch ε-Galactan. By G. O. Aspinall, E. L. Hirst and Else Ramstad. *J. Chem. Soc.*, 1958, 593-601.

36. Cereal Gums. Part III. The Constitution of an Araboxylan from Barley Flour. By G. O. Aspinall and R. J. Ferrier. *J. Chem. Soc.*, 1958, 638-642.

37. The Hemicelluloses of European Larch (*Larix decidua*). Part I. The Constitution of a Xylan. By G. O. Aspinall and J. E. McKay. *J. Chem. Soc.*, 1958, 1059-1066.

38. The Synthesis of 2-*O*-β-*D*-Xylopyranosyl-*L*-arabinose and its Isolation from the Partial Hydrolysis of Esparto Hemicellulose. By G. O. Aspinall and R. J. Ferrier. *J. Chem. Soc.*, 1958, in the press.

STRUCTURAL STUDIES ON POLYSACCHARIDES.

This thesis presents the results of a series of investigations directed towards a fuller understanding of the detailed molecular structure of polysaccharides. The major part of the published work deals with the chemistry of polysaccharides from the higher land plants, special attention being given to the hemicellulose group.

Polysaccharides may be classified either according to their biological source or function, or according to their chemical composition and structure. Cellulose, the principal cell-wall component of plants, provides an example of a polysaccharide with a clearly defined biological function. Polysaccharides providing the food reserve of the plant are typified by starch in a majority of land plants, while fructosans provide energy reserves in other plants, e.g. inulin in dahlia tubers and the levans in grasses. The gums exuded by some trees and shrubs are another easily recognisable group of polysaccharides, although there is at present no precise understanding of their biological function. The term hemicellulose is applied to those plant cell-wall polysaccharides which occur in close association with cellulose, especially in lignified tissues. Such a definition lacks precision in respect of both chemical structure and biological function, and in the following account these polysaccharides are

classified according to their main chemical features. In such a classification, for example, the term xylan will be used to denote polysaccharides containing a backbone of xylose residues, although the several polysaccharides of this group differ considerably in their more detailed structure, notably in the nature and number of other sugar residues which are also present and in the mode of attachment of these residues to the basal chains of the molecular structure. The classical definition of hemicelluloses was limited to those polysaccharides extracted from plants with alkaline reagents but not with water, and applied primarily to polysaccharides from lignified tissues. The chemical classification of xylans used here will also include some of the so-called cereal gums, which differ from the xylans of lignified tissues in solubility characteristics and probably also in biological function, but have common features in chemical structure.

Xylans.

The xylans are by far the most abundant polysaccharides of the hemicellulose group. They constitute 20-30% of the dry weight of agricultural residues such as corn cobs, cereal straws, and grain hulls. They are also the main hemicellulose components of hardwoods (20-25% of the wood) and occur in smaller but substantial amounts (7-12%) in softwoods together with glucomannans.

Most of the early work on the detailed structure of xylan

was carried out on the polysaccharide isolated from esparto grass (W.N.Haworth, E.L.Hirst and co-workers, J.C.S., 1929, 1739; 1934, 1917). It was clearly established that the main part of the molecule consisted of chains of 1:4-linked β-D-xylopyranose residues. This material, however, also contained some 5-10% of L-arabinose as a constituent and it was known that this sugar occurred, in part at least, in the furanose form. It was not known whether this sugar was present in an araboxylan or as a contaminating araban. More recently, further evidence of the structural complexity of esparto hemicellulose came from the work of S.K.Chanda, E.L.Hirst, J.K.N.Jones, and E.G.V.Percival (J.C.S., 1950, 1289), who showed that by repeated fractionation of this material it was possible to isolate a xylan devoid of arabinose residues. Definite evidence for the nature of the arabinose units in esparto hemicellulose came from a study of an arabinose-rich fraction (3). Methylation studies showed clearly that the majority of L-arabinose residues were present in the furanose form as non-reducing end groups, and that these could only arise from an araboxylan containing an essentially linear backbone of xylose residues and carrying side-chains linked to position 3 of xylose and terminated by L-arabofuranose residues. It is probable that these two molecular species present in esparto hemicellulose are representative of a whole series of closely related

polysaccharides which exist together in the plant. Later, evidence was found for still greater complexity in the structure of esparto xylans. During the preparation of xylobiose and xylotriose from the partial acid hydrolysis of esparto hemicellulose (17,25), a small amount of a disaccharide, characterised as 2-O-β-D-xylopyranosyl-L-arabinose (38), was isolated. It is probable, though not yet certain, that this disaccharide arises from a small proportion of xylopyranosylarabofuranose side-chains attached to the backbone of xylose residues in some fractions of esparto hemicellulose.

Polysaccharides of a similar complexity were encountered in investigations of wheat straw hemicellulose. In this case also it was possible to isolate a xylan devoid of arabinose residues (8). This xylan was composed of chains of 1:4-linked β-D-xylopyranose residues but also contained a small proportion of uronic acid (mainly glucuronic acid) residues as an integral part of the structure and present as single unit side-chains directly linked to the backbone. As in the case of esparto grass, wheat straw contains araboxylans in which the L-arabinose residues are present in the furanose form as terminal non-reducing groups (24). A very similar situation is found with the oat straw xylans. Fractionation of oat straw hemicellulose gave fractions containing varying proportions of arabinose residues, but it was not possible to isolate a xylan devoid of arabinose residues. A detailed

examination of one xylan fraction (20) showed that the backbone of xylose units carried a small proportion of L-arabofuranose and glucuronic acid units attached as side-chains. It was definitely established that the glucuronic acid side-chains were directly linked to position 2 of xylose, whereas the arabinose-containing side-chains were attached to position 3 of xylose.

Another xylan of the same general type was found in barley husks. Here, in addition to side-chains containing single arabinose or glucuronic acid residues, more complex xylosyl-arabinose units are present (30). The nature of these units was established from methylation studies on the polysaccharide and from the isolation of the disaccharide, 2-O-β-D-xylopyranosyl-L-arabinose, on partial acid hydrolysis of the polysaccharide. The optical rotation of this disaccharide was indicative of a β-glycosidic linkage, although other workers (Whistler and McGilvray, J. Amer. Chem. Soc., 1955, 77, 2212) had suggested that an α-linkage was present. Confirmation of the structure of the disaccharide and of the presence of a β-glycosidic linkage at the anomeric centre was obtained by synthesis (27, 38).

In contrast to the xylans of the Gramineae, which often contain appreciable proportions of arabinose residues and usually only small proportions of uronic acid residues, wood xylans contain 8-20% of acidic groups, and arabinose units

are either absent or only present in very small amount. In the present series of investigations three wood xylans have been examined. The first detailed structural studies were carried out on a beech xylan ("hemicellulose A") (9). This polysaccharide was shown to contain linear chains of approximately 70 1:4-linked β-D-xylopyranose units, with approximately every tenth xylose residue carrying a 4-O-methyl-D-glucuronic acid residue attached as a side chain to position 2 of xylose. A xylan fraction isolated from a typical softwood, Norway spruce, was shown to possess an essentially similar structure to that of the beech xylan, but having a somewhat greater chain length and a higher proportion of 4-O-methyl-D-glucuronic acid residues (23). Another xylan from a coniferous wood, European larch, was found to be very similar to the spruce xylan, except that in this case the backbone of xylose residues carried a small proportion of terminal L-arabofuranose residues attached as side chains in addition to the acidic groups (37).

Cereal grains contain small proportions of water-soluble polysaccharides (cereal gums) which may be separated into fractions rich in glucans (cf. barley β-glucan (10)) and rich in pentosans. Araboxylans from rye (32) and barley (36) have been studied, and structural investigations have shown that both polysaccharides belong to the same general family as the xylans from lignified tissues in containing linear chains of

1:4-linked xylose residues, but these xylans are characterised by the presence of a much higher proportion (30-40%) of terminal L-arabofuranose residues attached as single unit side-chains.

It is clear from these investigations and from those of workers in other laboratories [J.K.N.Jones (Kingston, Ontario), Adams, Bishop, and Perlin (Ottawa and Saskatoon), F.Smith (Minnesota), Whistler (Lafayette, Indiana), and Roudier (Paris)], to whom reference is made in several papers and in a review (21), that all the xylans from higher land plants, so far examined, contain essentially linear backbones of 1:4-linked β-D-xylopyranose residues to which the various side-chains containing L-arabinose and D-glucuronic acid residues are linked. Xylans from monocotyledenous plants are characterised by side-chains of L-arabinose, but in many cases D-glucuronic acid (sometimes as the 4-methyl ether) is also present. The xylans from dicotyledons and gymnosperms have side-chains of 4-O-methyl-D-glucuronic acid, but in some cases L-arabinose is also present. Thus, there is no marked structural division between the two groups of xylans. Furthermore, in both groups, the sugar residues attached as side-chains show the same preferred modes of linkage to the backbone, arabinose to position 3 and glucuronic acid to position 2 of xylose. It is probable that all these xylans are both polymolecular (containing polymer homologues with the same basic structure) and polydisperse (containing closely related molecular species differing in detailed structure). It is not possible at present to

indicate whether the various side-chains are attached to the backbone in a regular or in a random manner.

Since many polysaccharides of this group are isolated by extraction with alkaline reagents, a preliminary study has been made of the degradation of xylobiose and xylotriose (chosen as model compounds) by alkali (17, 25). The saccharinic acid formed as the main degradation product of 1:4-linked xylose derivatives has been characterised by the isolation of degradation products and by synthesis.

Mannans.

Mannose-containing polysaccharides occur in the seeds of many plants and in some hemicellulose fractions from coniferous woods. Two such mannans have been isolated from ivory nuts. These two materials differ in solubility characteristics, but earlier work by Klages (Annalen, 1934, 509, 159: 512, 185) indicated that both polysaccharides were composed of linear chains of 1:4-linked β-D-mannopyranose residues. The mannans A and B have now been re-examined using modern chromatographic techniques for the separation of the sugars and their derivatives (5, 33). The present results suggest that both fractions contain mixtures of polysaccharides and that mannan A and mannan B probably differ only in molecular size. The main conclusions from the earlier investigations have been confirmed, but other minor features of the molecular structure have been brought to light.

Until very recently there has been no detailed knowledge of the structures of the mannose-containing polysaccharides known to be present in coniferous woods. An examination of a glucomannan isolated from Sitka spruce wood (31) has indicated the presence therein of 1:4-linked β-D-mannopyranose and β-D-glucopyranose residues. Although it is not yet certain that the wood glucomannans are homogeneous, it is clear that at least part of the spruce hemicellulose is composed of a polysaccharide containing both mannose and glucose units.

Polysaccharides of considerable complexity occur in lichens. A preliminary study of the alkali-soluble polysaccharides from Reindeer moss (Cladonia alpestris) (12) has shown the presence of residues of D-mannose, D-glucose and D-galactose. Methylation studies showed that the majority of the D-galactose and some of the D-mannose and D-glucose residues occupy terminal positions, while chains of D-mannose and D-glucose residues constitute the backbone of the molecular structure.

Galactans.

In addition to the xylans and glucomannans which constitute the major hemicellulose fractions of coniferous woods, galactans or arabogalactans may be extracted with water. Larch woods are the most convenient source of such galactose-containing polysaccharides. A detailed study of the ε-galactan from European larch (35) has confirmed the general

conclusions from earlier investigations and new evidence has been presented concerning the order of linkage of the galactose residues in this highly branched polysaccharide. It is of particular interest that the backbone of 1:3-linked β-D-galactopyranose units, to which are attached side chains of 1:6-linked β-D-galactopyranose units, is also a feature of the molecular structure of gum arabic. The arabinose residues present in larch ε-galactan are now known to be present as a constituent part of an arabogalactan and do not arise from a separate araban.

Glucans.

Cellulose, which forms the basic cell-wall structure of the majority of land plants, is the most abundant natural polymer, and some of its fundamental chemistry has been reviewed (6). Rånby (Discuss. Faraday Soc., 1951, No. 11, 158) has shown that some of the resistant fragments, which result from the preferential acid hydrolysis of the amorphous regions of cellulose fibres, will peptise to form aqueous sols in the absence of electrolytes. Such cellulose sols have been prepared from esparto cellulose (18) and their average chain length has been determined by the methylation end group assay. The chemical accessibility of esparto cellulose, before and after acid degradation, has been determined by two methods.

As part of a general investigation of the polysaccharides of barley (10, 16, 30, 36) a detailed study has been made of

the starch isolated from malted barley (16). A comparison of the starches from barley and from the corresponding malted barley indicated that during malting the amylopectin undergoes preferential enzymic attack causing a shortening of the outer chains but with retention of the branched structure, whilst the amylose is relatively little degraded.

It was mentioned earlier that cereal grains contain small amounts of water-soluble polysaccharides, known as cereal gums. These may be separated into glucans (10) and araboxylans (32, 36). Barley is a convenient source of β-glucan (or β-glucosan). A structural study showed that this polysaccharide contains linear chains of β-D-glucopyranose residues with approximately equal proportions of 1:3- and 1:4-linkages (10).

The term callose is used to denote a group of membranous substances found in the phloem of plants. A typical example is the material which accumulates on the sieve plates of the phloem of the grape vine during winter dormancy and in the early stages of phloem senility. Although only very small quantities of this material were available for structural investigation, it was possible to show that callose from the grape vine is a polysaccharide resembling laminarin in being composed of chains of 1:3-linked D-glucose residues (29).

Fructosans.

In several plants fructosans replace starch as the carbo-

hydrate food reserve. These polysaccharides are characterised by 2:1-linked β-D-fructofuranose residues, as in inulin, or by 2:6-linkages, as in the grass levans; in some highly branched fructosans, e.g. irisin, both types of linkage are present. Many fructosans give, in addition to fructose, small amounts of glucose on hydrolysis, and in these studies particular interest attached to the role of this latter sugar in the polysaccharides. Typical of the levan series is the fructosan isolated from leafy cocksfoot grass (2). Methylation studies showed that this polysaccharide is composed of chains of *ca.* 25 2:6-linked β-D-fructofuranose residues. Virtually all the glucose was accounted for as non-reducing end groups, and the evidence suggested that the majority of fructosan chains are terminated by a glucose residue linked as in sucrose. The levan from perennial rye grass is another polysaccharide of very similar structure, and in this case conclusive evidence was found for the sucrose moiety. Fractionation of the oligosaccharides resulting from mild acid hydrolysis of ryegrass levan led to the isolation of sucrose as one of the products (1, 14). These experiments supported the evidence of other workers from enzymic studies (reviewed by Barker and Bourne, *Quarterly Rev.*, 1953, *7*, 56, and by Bacon, *Ann. Reports*, 1953, *50*, 281) that fructosans are built up in the plant from sucrose by transfructosylation.

Similar problems arise with respect to glucose residues

present in inulin. Previous methylation studies on inulin (Hirst, McGilvray, and Percival, J., 1950, 1297) indicated that glucose residues were present in two forms of combination, as non-reducing end-groups and linked through positions 1 and 3 in the middle of some of the chains. It has now been shown that such 1:3-linked glucose units are not present in inulin since the polysaccharide contains no glucose units resistant to attack by the periodate ion (4). It seems probable that the 2:4:6-tri-O-methyl-D-glucose previously isolated from the hydrolysis of methylated inulin arose from incomplete methylation of the polysaccharide.

Plant gums.

The plant gums exuded by many trees are polysaccharides of extreme complexity. Although no complete structure has yet been put forward for any of these polysaccharides, it is possible to compare those structural features of the plant gums which have been established with certainty (see ref. 21 for a brief review). The relationship of the chemical structure of gums to their botanical origin is a problem of especial interest, and the papers included here represent some of the first results of such an investigation. Several of the main features of the structure of gum ghatti have been established (15, 34), and it is already clear that this polysaccharide differs in important respects from the gums of the Acacia and Prunus genera. The gum exudate from Khaya grandifolia (19)

possesses an entirely different type of structure, and is unique amongst gums so far examined in containing two different uronic acid residues as constituents of the same polysaccharide. A preliminary examination of the gum from the related species Khaya senegalensis (19) showed that the same sugar residues are present but in different proportions. It is now known (from unpublished results) that K. senegalensis gum is heterogeneous; the major component, however, possesses a very similar structure to that of K. grandifolia gum.

Bacterial Polysaccharides.

Many micro-organisms form extracellular polysaccharides similar in their chemical complexity to the plant gums. Such a polysaccharide, elaborated by a strain of Aerobacter aerogenes, has been the subject of an investigation carried out in collaberation with Dr. J. F. Wilkinson. The composition of the extracellular polysaccharide was found not to change when the organism was grown with a variety of different sugars as sole carbon source (13). Some features of the molecular structure of the polysaccharide have been established (22).

Methyl Ethers of Sugars.

The methylation method is still one of the powerful methods for the determination of the mode of linkage of sugar residues in complex carbohydrates. The various methyl ethers

of sugars are therefore key reference compounds and the chemistry of the methyl ethers of D-mannose and of hexuronic acids has been reviewed (7, 11).

The syntheses of partially methylated sugars require the selective introduction of blocking groups. For some such syntheses it has been shown that advantage may be taken of differences in reactivity of equatorial and axial hydroxyl groups in compounds for which only one chair conformation is possible (26). Syntheses of some methyl ethers of D-mannose have been achieved in this way.

Periodate Oxidation.

The reactions of periodic acid and its salts are extensively used in structural studies of carbohydrates. A spectrophotometric method has been developed for the determination of periodate consumed during the oxidation of small quantities of carbohydrates (28).

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THE ISOLATION OF SUCROSE FROM THE PARTIAL HYDROLYSIS OF THE FRUCTOSAN FROM *LOLIUM PERENNE*

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The presence of small quantities of glucose in the hydrolysates of several fructosans has been reported by several groups of workers.[1–6] Some of these fructosans on methylation, followed by hydrolysis, have been found to yield methylated glucoses and in the case of the fructosan from leafy cocksfoot grass, all the glucose was isolated as 2 : 3 : 4 : 6-tetramethyl D-glucose.[4] To account for the presence of the glucose and also to explain the non-reducing properties of the fructosans it has been suggested that these polysaccharides consist of chains of fructofuranose residues terminated by a glucopyranose residue linked as in sucrose. Further support for this theory has come from investigations on the biosynthesis of fructosans, evidence having been brought forward to suggest that the fructosans are synthesized in nature, from sucrose by enzymic transfructosidation.[7]

It has been shown that fructosans[2, 3, 4] are degraded on heating in aqueous solution at 100° and that after several hours one of the degradation products travelled on the paper chromatogram at the same rate as sucrose and gave on further hydrolysis only glucose and fructose. We have carried out a large-scale "autohydrolysis" of the fructosan from perennial rye-grass.[3] Starting from 50 g. of polysaccharide the products from this treatment were separated first on charcoal columns[8] and secondly on cellulose columns[9] using as eluent benzene-*n*-butanol-pyridine-water (1 : 5 : 3 : 3; upper layer) to give glucose and fructose, a mixture of disaccharides and a mixture of higher oligosaccharides. The disaccharides were further separated on Whatman 3MM sheets using the same solvent.

By this means, *ca.* 300 mg. of a non-reducing disaccharide, giving on hydrolysis equal quantities of glucose and fructose, were obtained ($[\alpha]_D^{16}$ + 60, c = 2·4 in water; m.p. 184—185°, not depressed on admixture with an authentic specimen of sucrose). This material gave an *X*-ray powder photograph identical with that of pure sucrose, and was further characterized by conversion to the octacetate ($[\alpha]_D^{14°}$ + 60, c = 1·1 in chloroform; m.p. 72—73°, undepressed on admixture with an authentic specimen of sucrose octacetate).

A non-reducing trisaccharide, giving on hydrolysis two parts of fructose to one part of glucose, was also isolated in small quantity. This material, on graded hydrolysis in the presence of Amberlite resin 1R-100 and subsequent chromatographic examination, was shown to give rise to fructose and sucrose together with traces of glucose and a fructose-containing disaccharide.

These observations give additional support to the theory that fructosans contain terminal glucose residues linked as in sucrose. Further details of the use of "autohydrolysis" in the partial breakdown of fructosans will be published elsewhere in due course.

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66. *Studies on Fructosans. Part IV.* A Fructosan from* Dactylis glomerata.

By G. O. Aspinall, E. L. Hirst, (the late) E. G. V. Percival, and R. G. J. Telfer.

A fructosan from mid-season leafy cocksfoot grass (*Dactylis glomerata*) gave on hydrolysis D-fructose (97%) and D-glucose (3%). Methylation and hydrolysis yielded 1 : 3 : 4 : 6-tetramethyl D-fructose (4%), 2 : 3 : 4 : 6-tetramethyl D-glucose (1·8%), 1 : 3 : 4-trimethyl D-fructose (93·3%), and a dimethyl D-fructose (0·7%). The greater part of the material possesses therefore a molecular structure comprising a straight chain of *ca.* 25 2 : 6-linked fructofuranose residues terminated by a glucopyranose residue linked as in sucrose.

Fructosans of the levan type have been isolated from various plants such as rough-stalked meadow grass (*Poa trivialis*) (Challinor, Haworth, and Hirst, *J.*, 1934, 1560), barley leaves (Haworth, Hirst, and Lyne, *Biochem. J.*, 1937, **31**, 786), the roots of timothy-grass (*Phleum pratense*) (Schlubach and Sinh, *Annalen*, 1940, **544**, 101), and perennial rye-grass (*Lolium perenne*) (Laidlaw and Reid *). The present investigation has shown the fructosan from mid-season leafy cocksfoot grass (*Dactylis glomerata*) to possess a similar structure. Previous observations by Bell and Palmer (*Biochem. J.*, 1949, **45**, xiv) suggested a repeating unit of 14 residues for this fructosan, although physical measurements of sedimentation and diffusion constants suggested a chain length of 32—33. Palmer (*Biochem. J.*, 1951, **48**, 389), by estimation of the glucose produced on hydrolysis and on the basis of one glucose residue per chain, put forward values of 29 and 37 for the chain lengths of different samples of leafy-cocksfoot levan.

The cocksfoot grass used was oven-dried milled material prepared from grass cut in May, 1949, at the Jealott's Hill Agricultural Research Station. After preliminary extraction with ether and 80% aqueous methanol, the fructosan was extracted from the grass with water. Hydrolysis of the purified polysaccharide and analysis of the product by paper chromatography (Hirst and Jones, *J.*, 1949, 1659; Duff and Eastwood, *Nature*, 1950, **165**, 848) showed the presence of fructose (97%) and glucose (3%).

The fructosan was methylated under nitrogen with sodium hydroxide and methyl sulphate, and the product (OMe, 44·6%) was fractionated by dissolution in chloroform–light petroleum. Fraction 3 (OMe, 45·2%) was hydrolysed and the mixture of sugars separated on a cellulose column (Hough, Jones, and Wadman, *J.*, 1949, 2511). Two fractions (A and B) were obtained, corresponding to those previously observed on the paper chromatogram to be tetramethyl fructofuranose (containing some tetramethyl aldose) and a trimethyl fructose. Further elution yielded a small amount of a dimethyl fructose, which travelled at the same rate on the chromatogram as 3 : 4-dimethyl D-fructose. The tetramethyl fructofuranose was shown colorimetrically (cf. Arni and Percival, *J.*, 1951, 1822) to be present to the extent of *ca.* 4% in the hydrolysate. Its identity was confirmed by conversion into the crystalline tetramethyl D-fructofuronamide (Avery, Haworth, and Hirst, *J.*, 1927, 2313). Hypoiodite oxidation showed the presence of *ca.* 15% of tetramethyl aldose in A, and extraction of the remainder of the syrup with light petroleum gave a residual syrup from which some 2 : 3 : 4 : 6-tetramethyl D-glucose crystallised on seeding. Fraction B (93·3%) crystallised completely and proved to be 1 : 3 : 4-trimethyl D-fructose. Hypoiodite oxidation indicated at most only traces of aldose derivatives in this fraction.

In order to attempt a quantitative separation of tetramethyl glucose a further quantity of methylated fructosan was hydrolysed, and the resulting sugars converted into the

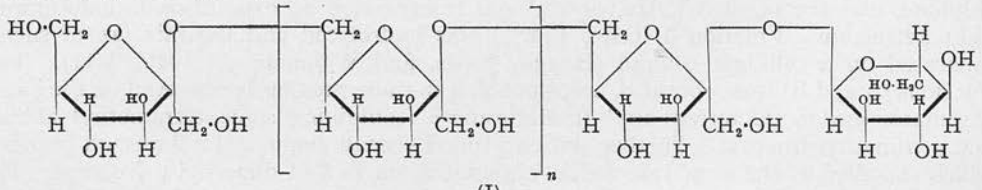
* Part III, *J.*, 1951, 1830.

corresponding glycosides. Extraction in a liquid-extractor with light petroleum (Brown and Jones, *J.*, 1947, 1344) gave only partial separation of tetramethyl methylfructoside from tetramethyl methylglucoside. Each fraction was hydrolysed and the free sugars were separated on cellulose columns. Complete separation of tetramethyl glucose from tetramethyl fructose was eventually achieved by partition chromatography. The tetramethyl aldose was identified as 2 : 3 : 4 : 6-tetramethyl D-glucopyranose, and the total quantities present in the various fractions were estimated by hypoiodite oxidation. The presence of a trace of trimethyl aldose in the trimethyl fructose fraction was shown both by hypoiodite oxidation and by paper chromatography, but the quantity (*ca.* 1 mg.) was without structural significance. A small quantity of a dimethyl fructose was also obtained from the column but this substance was not present in sufficient amount for complete identification.

From these experiments the proportions of the various methylated sugars produced on the hydrolysis of the methylated fructosan are tetramethyl fructofuranose 4·0%, tetramethyl glucopyranose 1·8%, trimethyl fructofuranose 93·3%, and dimethyl fructose 0·7%. The proportion of tetramethyl fructose indicates a chain of *ca.* 25 fructofuranose units in the fructosan. The quantity of dimethyl fructose is too small to be of structual significance and probably arises from undermethylation of the polysaccharide and/or demethylation during the hydrolysis. The quantity of tetramethyl glucose isolated, although not accounting for all the glucose present, does not permit of one residue per chain but suggests that a large proportion of fructosan molecules are terminated by a non-reducing glucose residue probably present in a sucrose-type linkage. It is possible that some degradation took place during the isolation and methylation of the polysaccharide with scission of some terminal glucose units. The absence of all but traces of other methylated glucoses eliminates the possibility of a contaminating glucosan's being present with the fructosan. Measurement of the molecular weight of the methylated fructosan by Barger's method (cf. Caesar, Gruenhut, and Cushing, *J. Amer. Chem. Soc.*, 1947, **69**, 617) gave a value corresponding to 17—25 $C_9H_{16}O_5$ units.

Oxidation of the polysaccharide consumed *ca.* 1·02 moles of sodium metaperiodate per $C_6H_{10}O_5$ residue, in agreement with the postulate of a molecule composed of fructofuranose residues linked through the 2 : 6-positions. Oxidation with potassium periodate (Brown, Halsall, Hirst, and Jones, *J.*, 1948, 27) yielded 1 mole of formic acid per 19—20 $C_6H_{10}O_5$ residues.

Heating the fructosan in water at 100° caused autohydrolysis. The fall in pH and the change in specific rotation were followed and samples were examined on the chromatograms as the reaction proceeded, spots being obtained corresponding to sucrose, glucose, fructose, and three unidentified acids. The " autohydrolysis " is at least in part a breakdown of the fructosan by acid hydrolysis.



(I)

These experiments essentially confirm the structure deduced from methylation data and show that this fructosan possesses a structure (I) very similar to that proposed for the fructosan from perennial rye-grass (Laidlaw and Reid, *loc. cit.*). The production of formic acid on periodate oxidation is slightly greater (1 mole per 19—20 $C_6H_{10}O_5$ residues) than would be expected if all the chains are terminated by a glucopyranose residue (1 mole per 25 residues). If, as has been suggested, scission of some of the terminal residues has occurred then the yield of formic acid from a reducing fructofuranose residue would explain the observed increase.

The structure of the fructosan as a chain of fructofuranose units linked through the 2 : 6-positions and terminated by a glucopyranose unit linked as in sucrose is in full agreement

with the theories put forward by Dedonder (*Compt. rend.*, 1950, **230**, 549, 997; 1951, **231**, 790; **232**, 1134, 1442) and by Bacon and Edelman (*Biochem. J.*, 1951, **48**, 114; **49**, 446, 529) that fructosans are built up in the plant from sucrose by enzymic trans-fructosidation.

Experimental

Preparation and Properties of the Polysaccharide.—Portions (100 g.) of mid-season cocksfoot grass (oven dried; H_2O, 9·8%; cut, May 26th, 1949) were extracted with ether (1 l.) for 5 hours. The residue was filtered and dried, then extracted with 80% aqueous methanol for a further 5 hours, after which the grass was filtered off and extracted for 3 hours with fresh 80% aqueous methanol (1 l.). The residue was removed, dried in air, and shaken with distilled water (1·3 l.) for 12 hours at room temperature. The grass was removed by filtration and washed with water, and the extract and washings (*ca.* 300 c.c.) were combined. The solution was heated to 93°, cadmium sulphate solution (40 c.c.; 10%) and sodium hydroxide solution (20 c.c.; 0·5N) were added, and the whole was kept at 95° for 3 minutes. After cooling, the cadmium hydroxide–protein complex was removed by filtration through " Filter Cel " (cf. Doak, *N.Z. J. Sci. Tech.*, 1939, **21**, 90B). The clear filtrate was de-ionised on columns of Amberlite resins (I.R.-100 and I.R.-4B), and the last traces of protein were removed by chloroform (0·25 vol.)–butanol (0·1 vol.) (Sevag, Lackmann, and Smollens, *J. Biol. Chem.*, 1938, **124**, 425). Evaporation of the neutral solution to 100 c.c. gave a light-amber coloured solution, which was poured into methanol. The colourless fructosan precipitated was removed at the centrifuge, washed twice with acetone, and dried in a vacuum-desiccator (P_2O_5 and paraffin wax). The polysaccharide was thus obtained as a fine colourless powder. By repetitions 30 g. of fructosan were prepared and combined. The average yield was *ca.* 3% of the dry weight of the grass. The fructosan had $[\alpha]_D^{15}$ −40·4° (*c*, 1·7 in H_2O) (Found: ash, 0·37; as sulphate, 0·45; N, 0·05%), and slowly reduced boiling Fehling's solution.

The fructosan (0·149 g.) was heated in 0·1N-oxalic acid (10 c.c.) at 70°, the following changes being observed: $[\alpha]_D^{15}$ −40·3° (0 min.); −46·3° (10 min.); −67·1° (20 min.); −78·5° (30 min.); −83·9° (40 min., const.). Paper chromatograms run in ethyl acetate–water–acetic acid (4 : 4 : 1 and 6 : 6 : 1) showed the hydrolysate to contain only fructose and a little glucose.

Hydrolysis of the polysaccharide until the rotation became constant ($[\alpha]_D^{16}$ −85° in 0·1N-oxalic acid), followed by neutralisation, filtration, de-ionisation, and concentration, gave a reducing solution which was shown chromatographically to consist of fructose *ca.* 97% and glucose *ca.* 3%. The fructose was estimated by oxidation with sodium metaperiodate as described by Hirst and Jones (*loc. cit.*), and the glucose by the Nelson colorimetric method (Nelson, *J. Biol. Chem.*, 1944, **153**, 375; Duff and Eastwood, *loc. cit.*). A synthetic glucose–fructose mixture containing 2·9% of glucose gave a recovery of 3·1% of glucose by this method.

Acetylation.—A specimen of polysaccharide (1·5 g.) was dissolved in dry pyridine (20 c.c.) according to the method of Pacsu and Mullen (*J. Amer. Chem. Soc.*, 1941, **63**, 1487). Acetic anhydride (20 c.c.) was added with stirring during 7 hours and the solution left for 2 days. The acetylated fructosan was precipitated with water (1 l.) and washed with water, and the product dried in a vacuum-desiccator ($CaCl_2$), giving a white powder. After reprecipitation from chloroform with light petroleum (b. p. 60—80°), a fine white powder was obtained {1·8 g.; $[\alpha]_D^{16}$ +22° (*c*, 1·1 in $CHCl_3$); $\eta_{sp.}^{20}/c' = 1·09$ where c' is the concn. in moles of the unit $C_{12}H_{16}O_8$ per l.} (Found: Ac, 43·1%).

To a solution of this acetyl derivative (0·5 g.) in chloroform (2·5 c.c.), cooled in a freezing mixture, a solution of sodium (0·25 g.) in absolute methanol (1 c.c.) was added (cf. Zemplen and Pacsu, *Ber.*, 1929, **62**, 1613). The mixture was shaken for 5 hours and ice-water (1 c.c.) added, followed by acetic acid (0·5 c.c.; 10%). Water (4 c.c.) was then added and the solution left to separate overnight. The regenerated fructosan was precipitated from the aqueous layer, with methanol. The product (0·22 g.) showed $[\alpha]_D^{15}$ −40·2° (*c*, 1·1 in H_2O) and mild acid hydrolysis followed by chromatographic examination showed the presence of fructose and glucose only.

Periodate Oxidation.—The fructosan (0·2864 g.) was dissolved in water (35 c.c.) and sodium metaperiodate (15 c.c.; 0·3M) added. The periodate uptake, determined by the arsenite method, was constant after 1 day at a value of 1·02 moles of periodate per $C_6H_{10}O_5$ residue.

Oxidation with potassium metaperiodate gave the following results (expressed as the number of $C_6H_{10}O_5$ residues per mole of formic acid liberated): 22·8 (73 hr.); 21·4 (100 hr.); 20·3 (126 hr.); 19·5 (171 hr., const.).

The phenylhydrazine hydrochloride–potassium ferricyanide colour reaction with the solution of periodate-oxidised polysaccharide was negative, indicating the absence of formaldehyde.

Methylation.—The fructosan (10 g.) was methylated in the usual way with methyl sulphate and sodium hydroxide solution under nitrogen at room temperature. After the first methylation, the partly methylated material was separated, dispersed in acetone, and further methylated with methyl sulphate and sodium hydroxide solution. The methylation in acetone was repeated twice, giving a product (9 g.) showing OMe 44·6%.

Fractionation of the methylated fructosan was effected by refluxing chloroform–light petroleum (b. p. 40—60°) mixtures of varying composition. The following results were obtained.

Fraction	% $CHCl_3$ in solvent	$[\alpha]_D^{15}$ (*c*, 1·1 in $CHCl_3$)	OMe, %	Wt. (g.)
1	27	−54°	45·3	0·4
2	30	−54	45·3	0·6
3	32·5	−56	45·2	4·5
4	35	−56	45·5	1·6

Fractions 1, 2, and 4, together with some unfractionated material, were combined and methylated with silver oxide and methyl iodide to give a product (Fr. X) which showed OMe, 45·4%, $[\alpha]_D^{16}$ −54·4° (*c*, 1·2 in chloroform).

Hydrolysis of Methylated Fructosan.—The methylated fructosan (Fr. 3 above, 3·209 g.) was heated with methanol (100 c.c.) and water (34 c.c.) containing oxalic acid (1·35 g.) at 80° for 24 hours, and a little insoluble material (0·187 g.) was filtered off. Water (300 c.c.) was then added gradually and the water–methanol mixture removed at 40° under diminished pressure. The solution was reduced to its original volume and heated at 80° for 5 hours, neutralised with calcium carbonate, and filtered, and the filtrate evaporated to small volume at 35° under diminished pressure. The mobile syrup was extracted several times with boiling chloroform, and the extracts were combined, dried (Na_2SO_4), and taken to small volume. Examination of the resultant pale yellow syrup on the paper chromatogram, with butanol–ethanol–water solvent and developing sprays of aniline oxalate and urea oxalate, showed the presence of four sugars, corresponding to tetramethyl fructofuranose, tetramethyl glucose, a trimethyl fructose, and a dimethyl fructose, the latter being in very low concentration (R_G : 1·00; 1·00; 0·84; 0·62).

The insoluble material from the above hydrolysis, on further hydrolysis, gave paper chromatograms identical with those described above.

Separation of Methylated Sugars.—The sugars arising from the hydrolysis of fraction 3 were separated on a cellulose column (70 × 3 cm.) with light petroleum (b. p. 100—120°)–butanol (7 : 3), saturated with water, as eluant (Hough, Jones, and Wadman, *loc. cit.*), to give Fractions A and B.

Fraction A contained tetramethyl fructofuranose and tetramethyl glucose only. The tetramethyl fructofuranose (110 mg.) was estimated colorimetrically (Arni and Percival, *loc. cit.*), and hypoiodite oxidation showed that tetramethyl glucose was present to the extent of 17 mg.

Fraction A (OMe, 49·4%; n_D^{20} 1·4500) showed $[\alpha]_D^{17}$ +39° (*c*, 0·99 in H_2O). *Ca.* 40 mg. of this fraction were oxidised with nitric acid and converted into the crystalline tetramethyl D-fructofuronamide, according to the method of Avery, Haworth, and Hirst (*loc. cit.*). After three recrystallisations from ether–light petroleum needles were obtained, of m. p. 99—101° (not depressed on admixture with an authentic specimen) (Found : C, 48·7; H, 7·7; OMe, 46·8. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·6; OMe, 49·8%).

The remainder of the syrup from fraction A was extracted with light petroleum (b. p. 35°), and both the extract and syrupy residue were taken to dryness. A speck of authentic crystalline tetramethyl glucose was added to the syrups, which were kept in a vacuum-desiccator over phosphoric oxide and paraffin wax, in a refrigerator for 3 weeks. The " extract " remained a syrup, but the " residue " crystallised partially. The crystals, separated on porous tiles, had m. p. 82°, not depressed on admixture with an authentic specimen of tetramethyl glucose.

Fraction B (2·211 g.) crystallised completely when kept at 0°. Chromatographic examination showed the absence of aldose. The sugar showed m. p. 73—75° after two recrystallisations from carbon tetrachloride–light petroleum, not depressed on admixture with authentic 1 : 3 : 4-trimethyl D-fructofuranose. It had $[\alpha]_D^{16}$ (*c*, 1·4 in H_2O) −27·4° (4 min.), −47·3° (20 min.), −56·6° (60 min.); −58·8° (5 hr.), −60·6° (20 hr.), −61·1° (68 hr., const.). (Found : C, 48·9; H, 8·4; OMe, 40·6. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·2; OMe, 41·9%). Examination by hypoiodite oxidation showed the absence of aldoses. Periodic acid oxidation (Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476) gave 0·8 mole of formaldehyde per mole of trimethyl sugar, estimated as the formaldehyde–dimedon compound (m. p. and mixed m. p. 186—187°).

A very small third fraction (*ca.* 7 mg.) was also obtained from the column, chromatographic examination of which showed the presence of only one sugar travelling at the same rate as 3 : 4-dimethyl fructose.

Hydrolysis of Methylated Fructosan X.—The methylated fructosan (2·512 g.), prepared as detailed above, was hydrolysed with methanolic oxalic acid as before; the solution was neutralised with calcium carbonate and filtered. The filtrate was concentrated, methanol added, and the water–methanol mixture distilled off with constant addition of methanol. The volume was taken to *ca.* 5 c.c., methanolic hydrogen chloride (50 c.c.; 0·3%) added, and the whole shaken, set aside at room temperature for 3 hours, neutralised, filtered, and freed from methanol as before. The aqueous solution (50 c.c.) was extracted, in the presence of a little barium carbonate, with purified light petroleum (b. p. 38—40°) in a liquid-extractor (Brown and Jones, *loc. cit.*) for periods of 10, 12, and 16 hours. The three extracts were evaporated practically to dryness and hydrolysed separately with sulphuric acid (15 c.c.; N/5) for 6 hours at 80°. The first contained only ketose, but the second and third contained also small quantities of tetramethyl aldose. The hydrolysed extracts were combined, to give fraction *a*. The aqueous solution in the extractor was filtered, taken to small volume, and hydrolysed with sulphuric acid (250 c.c.; N/5) for 6 hours at 80°, to give fraction *b*. Chromatographic examination of the products showed a high proportion of trimethyl fructose, with small amounts of tetramethyl fructose and tetramethyl glucose.

Separation of Fraction a.—The sugars in this fraction were separated into two fractions, a_1 and a_2, on a cellulose column (60 × 1·8 cm.) with light petroleum–butanol as before. Fraction a_1 contained tetramethyl fructofuranose (60 mg.) and tetramethyl glucose (13·4 mg.) estimated as described above. Fraction a_2 (0·228 g.) crystallised completely; hypoiodite oxidation indicated the presence of a small quantity of aldose, but aldoses could not be detected on a paper chromatogram, run in benzene–ethanol–water (167 : 45 : 15) (Andrews, Hough, and Jones, *J.*, 1952, 2746), it having been shown that 1 : 3 : 4-trimethyl fructose and 2 : 3 : 4-, 2 : 4 : 6-, and 2 : 3 : 6-trimethyl glucoses were separated under those conditions. No dimethyl sugars were obtained from fraction *a*.

Separation of Fraction b.—Separation of this fraction into its components was attempted by elution through a cellulose column (66 × 2·2 cm.) with benzene–ethanol–water, this solvent having been found capable of separating tetramethyl glucose from tetramethyl fructose on the paper chromatogram. Three main fractions, b_1, b_2, and b_3, were collected. Fraction b_1 contained tetramethyl glucose, tetramethyl fructose, and a little trimethyl fructose, separated on 4 sheets of 3 M.M. filter-paper by the above-mentioned solvent. The tetramethyl fructose, estimated colorimetrically, amounted to 30·6 mg., whilst alkaline hypoiodite showed the presence of 29·0 mg. of tetramethyl glucose. The trimethyl fructose amounted to 22 mg. Fraction b_2 (1·862 g.) was twice crystallised from carbon tetrachloride–light petroleum and the supernatant liquors from each crystallisation combined and examined on the paper chromatogram; a small quantity of trimethyl aldose was observed (*ca.* 1 mg. as determined by alkaline hypoiodite oxidation). Fraction b_3 was a mixture of trimethyl fructose and dimethyl fructose, with a trace of trimethyl glucose; these were separated on a cellulose column (56 × 1·6 cm.), elution with light petroleum–butanol (7 : 3) saturated with water as eluant and then with light petroleum–butanol (1 : 1) yielding trimethyl fructose, 36·0 mg., and dimethyl fructose, 16·4 mg.

Identification of Tetramethyl Glucose.—The aldose material from fraction b_1 after two crystallisations from ether–light petroleum (b. p. 40—60°) had m. p. 85°, not depressed on admixture with an authentic specimen of 2 : 3 : 4 : 6-tetramethyl D-glucose, $[\alpha]_D^{15}$ +95° ⟶ +84° (*c*, 0·5 in H_2O) (Found: OMe, 51·4%).

Examination of Dimethyl Fructose.—This had R_G 0·61 [butanol–ethanol–water (40 : 10 : 50)], OMe, 27·2%, and n_D^{20} 1·4784.

Molecular Weight by Barger's Method.—Barger's capillary technique (*loc. cit.*) was employed to determine the molecular weights of the acetylated and methylated derivatives of the fructosan. Droplets of solutions of known concentration were compared with solutions of sucrose octa-acetate (1—6 × 10^{-3}M). No satisfactory results could be obtained with 4%, 2%, and 1% solutions of the acetate, but a 1% solution of the methylated fructosan (fraction 3) gave reproducible values between 3440 and 5160, the equilibrium point being found to be between concentrations of sucrose octa-acetate of 2 × 10^{-3} and 3 × 10^{-3}M. An attempt to increase the accuracy of the method by employing a 2% solution of the methylated fructosan was unsuccessful.

Autohydrolysis of the Fructosan.—The fructosan (0·184 g.) in distilled water (25 c.c.) showed the changes at 100°: $[\alpha]_D^{15}$ −40·1° (initial value), −37·5° (4·5 hr.), −36·7° (9 hr.), −34·3° (14 hr.), −42·4° (19 hr.), −48·9° (22 hr.), −84·4° (29 hr.), −86·2° (35 hr.). After 22 hours a portion (15 c.c.) of the solution was chromatographically examined. It contained fructose, glucose, and three oligosaccharides, one of which travelled at the same rate as sucrose. Elution of this sugar from the paper, followed by hydrolysis, gave a solution containing two sugars, which travelled on the paper chromatogram at the same rate as glucose and fructose respectively.

After 30 hours, the pH of the solution was 3·4 (initial value 6·1) owing to the formation of three acids, observed chromatographically on running samples of the solution in butanol saturated with 1·5N-ammonia (Reich and Lederer, *Biochem. J.*, 1951, **50**, 60) and developing with methyl-red–methylene-blue (Conway and Byrne, *Biochem. J.*, 1933, **27**, 419).

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329. *The Hemicelluloses of Esparto Grass* (Stipa tenacissima, *L.*). *The Arabinose-rich Fraction.*

By G. O. Aspinall, E. L. Hirst, R. W. Moody, and (the late) E. G. V. Percival.

An arabinose-rich fraction has been isolated from esparto-grass hemicellulose. This gave on hydrolysis D-xylose (12 parts), L-arabinose (5 parts), D-glucose (1 part), and D-galactose (1 part). Hydrolysis of the methylated polysaccharide gave 2 : 3 : 5-trimethyl L-arabinose (1 part), 2 : 3-dimethyl D-xylose (3 parts), and 2-methyl D-xylose (1 part), together with smaller quantities of 2 : 3-dimethyl L-arabinose, 2 : 3 : 4 : 6-tetramethyl D-galactose, 2 : 4 : 6-trimethyl D-galactose, and 2 : 4-dimethyl D-galactose. It is concluded that the majority of the L-arabofuranose residues are present as side-chains attached to a main chain of 1 : 4-linked D-xylopyranose residues.

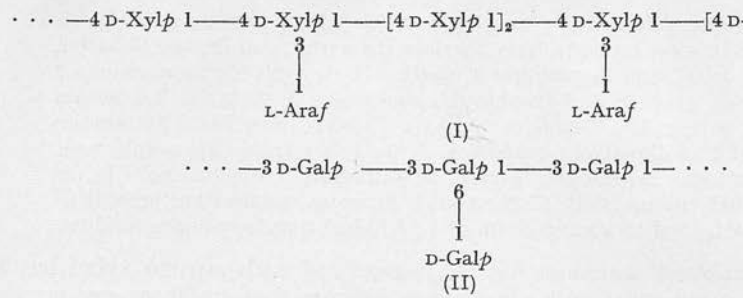
The presence of combined arabinose (*ca.* 7%) associated with esparto xylan has been known for some time and early work appeared to indicate that arabinose was present in the furanose form as an end group in a molecule comprising 18—20 xylopyranose residues (Haworth, Hirst, and Oliver, *J.*, 1934, 1917). More recently it was found that, by repeated fractional precipitation as the copper complex under carefully controlled and very mild conditions, a xylan devoid of arabinose residues could be obtained from esparto (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289). This polysaccharide was shown to consist of a singly branched molecule containing 75($\pm$5) D-xylopyranose units. These residues are linked through $C_{(1)}$ and $C_{(4)}$ except that the single branching point is formed by a 1 : 3-union. The mode of occurrence of the arabinose residues in the hemicellulose fraction remained obscure and the present investigation was undertaken to obtain fuller information on this point. Possibilities which could be envisaged were that the arabinose residues might be present in an associated araban of the type found in pectic substances (Hirst and Jones, *J.*, 1939, 452) or in a xyloaraban or araboxylan such as that recently isolated from wheat flour (Perlin, *Cereal Chem.*, 1951, **28**, 352). In the latter instance the arabofuranose residues were attached as side chains to a main chain of 1 : 4-linked D-xylopyranose units.

Crude esparto xylan was extracted from delignified esparto grass by the method of Chanda *et al.* (*loc. cit.*), and the hemicellulose thus obtained was extracted with hot 70% aqueous alcohol. This gave an arabinose-rich fraction, which on hydrolysis yielded xylose (12 parts), arabinose (5 parts), glucose (1 part), and galactose (1 part). Although many methods of extraction and fractionation were investigated the separation of an araban in a pure state was not achieved and we regard it as doubtful whether such a polysaccharide composed solely of arabinose residues does in fact occur in esparto hemicellulose.

The material richest in arabinose residues (hereinafter called polysaccharide A) was methylated, first by Fear and Menzies's method (*J.*, 1926, 937) and then with methyl iodide and silver oxide. The methylated derivative (OMe, 36·6%) was hydrolysed successively with methanolic and with aqueous hydrochloric acid, and the products of hydrolysis were chromatographed on a cellulose column. Although complete separation of all the fractions was not achieved, the presence of the following sugars was established by the isolation of crystalline derivatives (quantitative analyses indicated that these were present in the proportions shown in parentheses) : 2 : 3 : 5-trimethyl L-arabinose (29), 2 : 3 : 4 : 6-tetramethyl D-galactose (4), 2 : 3-dimethyl D-xylose (100), 2 : 3-dimethyl L-arabinose (6), 2 : 4 : 6-trimethyl D-galactose (8), 2-methyl D-xylose (34), and 2 : 4-dimethyl D-galactose (8). The trimethyl pentose fraction was shown chromatographically to yield on demethylation a small quantity of xylose in addition to arabinose. It is likely therefore that the fraction contained, as is to be expected, a little 2 : 3 : 4-trimethyl xylose but attempts to form derivatives were unsuccessful. No methylated derivatives of glucose were isolated.

The small proportion of 2 : 3-dimethyl arabinose present together with the complete

absence of a monomethyl arabinose preclude the existence of a multi-branched araban of the type encountered in the pectic arabans. The proportions of the main constituents of the mixture of methylated sugars indicate that most of the arabofuranose residues must exist as side chains to a backbone of D-xylopyranose units, such side chains being attached to approximately every fourth xylose residue through position 3, as in (I). Owing to the incomplete methylation of the polysaccharide A it is impossible to ascribe definite structural



[For conventional symbols see *J.*, 1952, 5251.]

significance to the isolation of the small quantity of dimethyl arabinose. It is difficult also to account unambiguously for the methylated galactoses present. Although these residues could arise from a mixed polysaccharide, it is equally possible that a branched galactan exists as a separate entity, for which a possible repeating unit (II) is suggested above. The numerical results show, however, that many of the arabinose residues must have been attached to xylose residues and we conclude that there is present in the hemicellulose fraction of esparto an araboxylan of a type closely resembling the material discussed by Perlin (*loc. cit.*) and containing a main chain of 1 : 4-linked xylose residues to which L-arabofuranose residues are attached as side chains. The composition of this hemicellulose fraction is therefore extremely complex, much of the xylan being present, as previously shown by Chanda *et al.* (*loc. cit.*), as a true xylan containing no arabinose residues.

Experimental

Preparation of Polysaccharide A.—Crude esparto xylan was extracted from delignified esparto grass by the procedure of Chanda *et al.* (*loc. cit.*). The crude xylan (300 g.) was successively extracted four times for 24 hr. in batches (30 g.) with boiling aqueous alcohol (1 l.; 70% v/v), the insoluble material being removed by filtration of the hot extract. The filtrate was concentrated under diminished pressure, acidified with acetic acid, and poured into acetone (10 vols.). The collected precipitate (A) was washed successively with alcohol and ether and dried in a vacuum-desiccator (P_2O_5), to yield a fawn-coloured powder (35 g.; ash, 3·5%). Chromatographic examination of the hydrolysate (Hirst and Jones, *J.*, 1949, 1659) showed the presence of xylose (12 parts), arabinose (5 parts), glucose (1 part), and galactose (1 part).

Methylation of Polysaccharide A.—The polysaccharide A (30 g.) was converted into its thallium derivative and methylated twice by Fear and Menzies's method (*loc. cit.*) and four times more with silver oxide and methyl iodide, giving a crisp brown glass (9·3 g.; OMe, 36·6%), purified by dissolution in hot acetone to give methylated polysaccharide A (7·5 g.; OMe, 36·6%; ash, 0·1%).

Hydrolysis of Methylated Polysaccharide A.—The methylated polysaccharide A (7·5 g.) was refluxed with methanolic hydrogen chloride (380 c.c.; 1%) for 12 hr. (constant rotation). At the end of this time the methanolic hydrogen chloride was removed under diminished pressure and the resultant syrup hydrolysed on the water-bath with hydrochloric acid (500 c.c.; 0·5N) for 8 hr. (constant rotation). An insoluble residue (0·42 g.; OMe, 18·0%) was removed at the centrifuge, the clear solution was neutralised with silver carbonate and filtered, and the silver salts were removed with hydrogen sulphide. The solution was finally deionised with Amberlite resins IR-100 and IR-4B, and concentrated to a syrup. Paper-chromatographic examination of the syrup showed the presence of a trimethyl pentose (R_G 0·95), 2 : 3-dimethylxylose, 2 : 3-dimethyl arabinose, a monomethyl pentose (R_G 0·38), and traces of free pentose.

Separation of Methylated Sugars.—The syrupy hydrolysate (5·5 g.) was fractionated on a cellulose column (90 × 3·4 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511), elution being carried out with (*a*) light petroleum (b. p. 100—120°)–butanol (7 : 3), saturated with water, (*b*) light petroleum–butanol (1 : 1), saturated with water, and (*c*) butanol, partly saturated with water. The fractions obtained (see Table) were examined by paper chromatography with butanol–ethanol–water–ammonia (40 : 10 : 49 : 1; top layer) as solvent, and in a number of cases the sugars produced after demethylation were examined (Hough, Jones, and Wadman, *J.* 1950, 1702).

Analysis of hydrolysate of methylated polysaccharide A.

Fraction	Wt. of material eluted (g.)	$[\alpha]_D^{16}$	Paper chromatography		Sugar given after demethylation *
			R_G *	Sugar	
1	0·053	—	1·00 (t)	Tetramethyl glucose	—
			0·95	Trimethyl pentose	—
2	0·265	−26·8°	0·95	Trimethyl pentose	Arabinose, xylose (trace), glucose (trace)
3	0·307	−18·3	0·95	Trimethyl pentose	Arabinose, xylose (trace)
4	0·023	+23	0·95	Trimethyl pentose	—
			0·88	Tetramethyl galactose	—
5	0·056	+79·2	·88	Tetramethyl galactose	—
6	0·116	−18·2	0·88 (t)	Tetramethyl galactose	Galactose (trace)
			0·82	Unknown	Arabinose
			0·74 (t)	Dimethyl xylose	Xylose (trace)
7	0·068	+30·8	0·82	Unknown sugar	—
			0·74	Dimethyl xylose	—
8	1·907	+30·0	0·74	Dimethyl xylose	—
9	0·063	+46	0·74	Dimethyl xylose	Xylose, galactose
			0·70	Trimethyl galactose	
10	0·065	+54·7	0·74 (t)	Dimethyl xylose	—
			0·70	Trimethyl galactose	—
			0·64 (t)	Dimethyl arabinose	—
11	0·209	+85·2	0·70	Trimethyl galactose	—
			0·64	Dimethyl arabinose	—
12	0·103	—	0·64 (t)	Dimethyl arabinose	—
			0·38	Monomethyl xylose	—
13	0·559	+34	0·38	Monomethyl xylose	—
14	0·043	—	0·38	Monomethyl xylose	—
			0·36	Dimethyl galactose	—
15	0·119	+71·6	0·36	Dimethyl galactose	Galactose
16	0·067	—	0·36	Dimethyl galactose	—
			0·30 (t)	Unknown	—
17	0·104	—	0·15	Xylose	—
			0·13 (t)	Arabinose	—

* t = trace.

Identification of fractions. 2 : 3 : 5-Trimethyl L-arabinose was identified in fractions 2 and 3 by conversion into 2 : 3 : 5-trimethyl L-arabonamide (m. p. and mixed m. p. 135—136°). An attempt to prepare the aniline derivative of 2 : 3 : 4-trimethyl D-xylose from fraction 3 was unsuccessful. 2 : 3 : 4 : 6-Tetramethyl D-galactose was identified in fraction 5 by conversion into 2 : 3 : 4 : 6-tetramethyl D-galactosylaniline (m. p. and mixed m. p. 194—195°). 2 : 3-Dimethyl D-xylose was identified in fraction 8 by conversion into 2 : 3-dimethyl D-xylosylaniline (m. p. and mixed m. p. 120—122°) and 2 : 3-dimethyl D-xylonamide (m. p. and mixed m. p. 131—132°). 2 : 4 : 6-Trimethyl D-galactose and 2 : 3-dimethyl L-arabinose were identified in fraction 11 by conversion into 2 : 4 : 6-trimethyl D-galactosylaniline (m. p. and mixed m. p. 165—166°) and 2 : 3-dimethyl L-arabonamide (m. p. and mixed m. p. 159—160°) respectively. 2-Methyl D-xylose from fraction 13 crystallised completely from acetone (m. p. and mixed m. p. 134—136°) and was converted into 2-methyl D-xylosylaniline (m. p. and mixed m. p. 124—125°). 2 : 4-Dimethyl D-galactose from fraction 15 was recrystallised from acetone containing 1% of water, giving the monohydrate (m. p. and mixed m. p. 93—96°), and was characterised as 2 : 4-dimethyl D-galactosylaniline (m. p. and mixed m. p. 209—210°) and 2 : 4-dimethyl D-galactonamide (m. p. and mixed m. p. 163—165°).

Quantitative Estimation of Methylated Sugars.—After identification of the fractions obtained from the column the relative proportions of the different sugars were calculated, the results being tabulated below. In the case of fractions containing mixtures of sugars the relative proportions were estimated approximately from the intensities and colours of the spots given on paper chromatograms.

Sugar	Wt. (g.)	Parts per 100 parts of 2 : 3-di-methyl xylose	Sugar	Wt. (g.)	Parts per 100 parts of 2 : 3-di-methyl xylose
Trimethyl pentose	0·636	32	Trimethyl galactose ...	0·160	8
Tetramethyl galactose	0·080	4	Monomethyl xylose ...	0·680	34
Dimethyl xylose	2·000	100	Dimethyl galactose ...	0·160	8
Dimethyl arabinose ...	0·120	6			

On the assumption that trimethyl xylose was present in the same quantity as in the hydrolysate of methylated arabinose-free xylan, 32 parts of trimethyl pentose would contain 3 parts of trimethyl xylose and 29 parts of trimethyl arabinose.

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THE OCCURRENCE OF GLUCOSE RESIDUES IN INULIN

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The occurrence of small quantities of glucose in the hydrolysates of inulins from various sources has been reported on several occasions.[1, 2, 3] As in the case of the grass levans[4, 5, 6] it has been suggested that the inulin molecule consists of a chain of fructofuranose residues terminated by a non-reducing glucopyranose residue linked as in sucrose, and biosynthetic studies have provided evidence that fructosans are synthesized in nature from sucrose by enzymic transfructosidation.[7] Hirst, McGilvray and Percival,[1] however, in their studies on the inulin from the Blue Danube variety of dahlia, isolated from the hydrolysis of the methylated polysaccharide a significant amount of 2 : 4 : 6-trimethyl D-glucose in addition to the 2 : 3 : 4 : 6-tetramethyl D-glucose arising from the terminal glucose residue, and suggested the possibility of a second glucopyranose residue linked through $C_{(1)}$ and $C_{(3)}$ at some undetermined position in the chain. Such a glucose residue in inulin would not be attacked by sodium periodate solution[8] and the reaction of inulin with periodate has been further investigated.

The sample of inulin, prepared from dahlia tubers (Crimson Flag variety), had $[\alpha]_D^{15}$ −40·3 and gave on hydrolysis fructose (97·2%) and glucose (2·8%). The inulin was oxidized with sodium metaperiodate solution (0·1M) for five days and the insoluble periodate-oxidized inulin separated and hydrolyzed with aqueous methanolic hydrogen chloride. Chromatographic examination of the hydrolysate showed the complete absence of glucose under conditions in which the amount of glucose estimated to arise from a 1 : 3-linked glucose residue in inulin would be readily detected.

This result shows, therefore, that inulin does not contain a 1 : 3-linked glucopyranose residue, and it seems probable, in view of the known difficulty of obtaining complete methylation of sucrose,[9] that the 2 : 4 : 6-trimethyl D-glucose arose from incomplete methylation of the terminal glucose residue of the polysaccharide. Bell and Palmer[3] also obtained "trimethyl glucose" (a mixture of sugars with the 2 : 4 : 6-isomer predominating) from the hydrolysis of the methylated inulin from *Inula helenium*, further indicating the difficulty of obtaining complete methylation of inulin.

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635. *The Mannans of Ivory Nut* (Phytelephas macrocarpa). *Part I. The Methylation of Mannan A and Mannan B.*

By G. O. Aspinall, E. L. Hirst, (the late) E. G. V. Percival, and I. R. Williamson.

Mannans A and B from ivory nut (*Phytelephas macrocarpa*) gave on hydrolysis D-mannose (97·6%), D-galactose (1·8%), and D-glucose (0·8%), and D-mannose (98·3%), D-galactose (1·1%), and D-glucose (0·8%), respectively. Hydrolysis of methylated mannan A gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose (7·3%), 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (1·7%), 2 : 3 : 6-tri-*O*-methyl-D-mannose (83·0%), 2 : 3 : 4-tri-*O*-methyl-D-mannose (6·8%), and a di-*O*-methyl-D-mannose (1·2%). Hydrolysis of methylated mannan B gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose (1·3%), 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (1·3%), 2 : 3 : 6-tri-*O*-methyl-D-mannose (81·8%), 2 : 3 : 4-tri-*O*-methyl-D-mannose (14·3%), and a di-*O*-methyl-D-mannose (1·3%). 2 : 3 : 4-Tri-*O*-methyl-D-mannose was isolated from both hydrolysates as the non-reducing disaccharide 2 : 3 : 4-tri-*O*-methyl-D-mannopyranosyl 2 : 3 : 4-tri-*O*-methyl-D-mannopyranoside. It is concluded that mannan A and mannan B both contain at least two types of molecule, one terminated by a D-mannopyranose residue and the other terminated by a D-galactopyranose residue. The majority of the mannopyranose residues are linked through positions 1 and 4 but 1 : 6-linkages are also present. Mannans A and B differ only in molecular size having average chain lengths of 10—13 and 39—40, respectively.

The chemistry of the endosperm of ivory nut (*Phytelephas macrocarpa*) was first studied by Reiss (*Ber.*, 1889, **22**, 609) who obtained on hydrolysis a sugar that was subsequently identified as mannose by Fischer and Hirschberger (*Ber.*, 1889, **22**, 1155). Several investigators (Johnson, *J. Amer. Chem. Soc.*, 1896, **18**, 214; Baker and Pope, *J.*, 1900, **77**, 676; Ivanov, *Journ. f. Landw.*, 1908, **56**, 217; Pringsheim and Seifert, *Z. physiol. Chem.*, 1922, **123**, 205) studied the polysaccharide extracted from ivory nuts with alkali and showed that it yielded mainly mannose on hydrolysis. Patterson (*J.*, 1923, 1139) methylated the polysaccharide and from the hydrolysate isolated a syrupy trimethylmannose which could be quantitatively converted into crystalline methyltetra-*O*-methyl-α-D-mannoside. Patterson concluded, therefore, that all the mannose residues were of the ordinary stable type.

Further investigations by Lüdtke (*Annalen*, 1927, **456**, 201) showed that two different mannans could be obtained from delignified ivory nuts by dissolution in cuprammonium solution followed by fractional precipitation with sodium hydroxide. The mannan mixture was separated into two fractions, mannan A (soluble in aqueous sodium hydroxide) and mannan B (insoluble in aqueous sodium hydroxide).

Methylation studies of mannans A and B were carried out by Klages (*Annalen*, 1934, **509**, 159; **512**, 185) who concluded that mannan A consisted of a chain of 80 1 : 4-linked β-D-mannopyranose residues and postulated that mannan B was similarly constituted. More recent investigations by Ward (M.Sc. Thesis, Manchester, 1947) indicated that mannan A had a repeating unit of 15 and that the 1 : 4-linkage was not the only linkage present. The present investigation was undertaken to determine whether the two mannans were similarly constituted and with the aid of modern chromatographic techniques to determine the fine structure of each.

Mannan A was extracted from delignified ivory nut shavings with cold aqueous potassium hydroxide and purified by two precipitations, as the copper complex, with Fehling's solution. Hydrolysis of the mannan, $[\alpha]_D^{15}$ −46° (*c*, 0·7 in N-NaOH), gave D-mannose

Fraction 1. The syrup (242 mg.) was partly crystalline and after recrystallisation from ether had m. p. 109—111°. The mixture of syrup and crystals had $[\alpha]_D^{20}$ +23° ⟶ +10° (*c*, 0·7 in H_2O) (Found : OMe, 50·4. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). Chromatographic examination showed only 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose, and the aniline derivative had m. p. 144—145° (not depressed on admixture with an authentic sample) and $[\alpha]_D^{18}$ −94° ⟶ −42° (*c*, 0·8 in $COMe_2$) (Found : C, 61·8; H, 8·0. Calc. for $C_{16}H_{25}O_5N$: C, 61·7; H, 8·0%). A portion of the syrup was converted into the δ-lactone {$[\alpha]_D^{15}$ +148° (*c*, 1·0 in H_2O)} which was characterised by conversion into 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannonic acid phenylhydrazide, m. p. and mixed m. p. 185—186° (Found : N, 7·8. Calc. for $C_{16}H_{26}O_6N_2$: N, 8·2%).

Fraction 2. The syrup (58 mg.) was chromatographically pure and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 195—196°, $[\alpha]_D^{16}$ −136° (*c*, 0·4 in C_5H_5N), $[\alpha]_D^{14}$ −76° (*c*, 0·3 in $COMe_2$).

Fraction 3. The syrup (2·623 g.) had $[\alpha]_D^{13}$ −7° (*c*, 4·6 in H_2O) (Found : OMe, 38·6. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Chromatographic examination showed only 2 : 3 : 6-tri-*O*-methylmannose, and hypoiodite oxidation indicated 92% purity. A sample was converted into 2 : 3 : 6-tri-*O*-methyl-*N*-phenyl-D-mannosylamine, m. p. and mixed m. p. 127—128°. A second sample was converted into the γ-lactone, m. p. and mixed m. p. 81—82°, $[\alpha]_D^{16}$ +64·5° (*c*, 3·2 in H_2O), part of which was converted into 2 : 3 : 6-tri-*O*-methyl-D-mannonic acid phenylhydrazide, m. p. and mixed m. p. 132—133°.

Fraction 4. Paper chromatography showed the presence of two sugars, and the syrup (240 mg.) was separated on Whatman 3MM paper with butanol–ethanol–water (4 : 1 : 5; top layer) as eluant to give fractions (4*a*) (206 mg.) and (4*b*) (34 mg.). Fraction 4*a*, obtained as a syrup, travelled on the chromatogram as a trimethylhexose but at a speed different from those of 2 : 3 : 6- and 3 : 4 : 6-tri-*O*-methylmannoses. After several weeks the syrup crystallised almost completely to a non-reducing *substance X*, m. p. 148—150°, $[\alpha]_D^{18}$ +55° (*c*, 1·4 in H_2O, unchanged after 48 hr.), $[\alpha]_D^{18}$ +65° (*c*, 0·7 in 1% methanolic hydrogen chloride, unchanged after 100 hr.) (Found : C, 50·5; H, 7·9; OMe, 42·9. $C_{18}H_{34}O_{11}$ requires C, 50·7; H, 8·0; OMe, 43·7%). Demethylation (Hough, Jones, and Wadman, *J.*, 1950, 1702) showed *X* to be a mannose derivative, and the molecular weight, obtained by Barger's isopiestic method (cf. Caesar, Gruenhut, and Cushing, *J. Amer. Chem. Soc.*, 1947, **69**, 617), corresponded to that of a hexamethyldisaccharide.

Fraction 4*b*, which remained as a syrup, was shown by demethylation to be a mannose derivative and travelled on the chromatogram at the same rate as 2 : 3-di-*O*-methyl-D-mannose.

Substance *X* (60 mg.) was methylated with methyl iodide and silver oxide, and the product (69 mg.) was hydrolysed on the water-bath with sulphuric acid (1 c.c.; N). The hydrolysate was neutralised with Amberlite resin IR-4B and concentrated to a syrup (60 mg.). Chromatographic examination showed only tetramethylmannose, and the syrup was converted into 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-mannosylamine (70 mg.), m. p. and mixed m. p. 144—145°.

Hydrolysis of *X* gave a chromatographically pure trimethylmannose which was oxidised by lead tetra-acetate (cf. Buchanan, Dekker, and Long, *J.*, 1950, 3162). Oxidation with sodium metaperiodate solution liberated formaldehyde, which was detected with phenylhydrazine hydrochloride, potassium ferricyanide, and concentrated hydrochloric acid (cf. Chanda, Hirst, Percival, and Ross, *loc. cit.*). The trimethylmannose was therefore 2 : 3 : 4-tri-*O*-methyl-D-mannose, and X was 2 : 3 : 4-*tri*-O-*methyl*-D-*mannopyranosyl* 2 : 3 : 4-*tri*-O-*methyl*-D-*mannopyranoside*.

Quantitative Examination of the Methylated Sugars.—A portion of the hydrolysate of methylated mannan A was separated chromatographically (Hirst, Hough, and Jones, *J.*, 1949, 298), benzene–ethanol–water (169 : 47 : 15; top layer) being used as solvent, and the tetramethyl and trimethyl sugars estimated by alkaline hypoiodite (cf. Chanda, Hirst, Jones, and Percival, *loc. cit.*) [Found (results expressed as c.c. of 0·01N-iodine consumed) : "tetra," 0·16, 0·41; "tri," 1·43, 3·68]. These figures correspond to a molecular ratio of tetramethyl sugars : trimethyl sugars of 1 : 9.

Mannan B

Preparation of Mannan B.—The residue remaining after extraction of mannan A was extracted with potassium hydroxide solution (14%) to remove further mannan A, washed with water to remove alkali, and shaken in the dark with cuprammonium solution to which sucrose had been added to minimise oxidation of the polysaccharide. After removal of undissolved materials at the centrifuge, sodium hydroxide solution was added until the mixture was 0·2N

with respect to sodium hydroxide. The bulky complex which separated was stirred with water and decomposed with glacial acetic acid, the mannan being precipitated by the addition of an equal volume of ethanol. The material thus obtained was subjected to a second precipitation by the same procedure and crude mannan B isolated. Chromatographic examination of the hydrolysate showed the presence of mannose, glucose, and galactose. Further purification was effected by re-extraction with potassium hydroxide solution (7%) followed by dissolution in anhydrous formic acid (Dr. J. K. N. Jones, personal communication), mannan B {$[\alpha]_D^{15}$ $-26°$ (*c*, 0·8 in anhydrous formic acid, unchanged after 70 hr.)} being obtained by precipitation with ethanol. Chromatographic examination of the hydrolysate showed the presence of mannose (98·3%), galactose (1·1%), and glucose (0·8%).

Methylation of Mannan B.—Mannan B (11 g.) was methylated 12 times with methyl sulphate and sodium hydroxide and twice with methyl iodide and silver oxide to give a product (3·5 g.; Found: OMe, 44·9%) isolated by dissolution in hot chloroform. Fractionation by dissolution in chloroform–light petroleum mixtures gave a main fraction {2·8 g.; $[\alpha]_D^{15}$ $-20°$ (*c*, 1·0 in $CHCl_3$); Found: OMe, 45·0%}.

Hydrolysis of Methylated Mannan B and Separation of Methylated Sugars.—Methylated mannan B (2·5 g.) was hydrolysed successively with anhydrous formic acid (15 c.c.) and sulphuric acid (7 c.c.; N) as described for methylated mannan A. The hydrolysate (2·0 g.) was fractionated on cellulose as described previously and four fractions collected.

Fraction 1. Chromatographic examination of the syrup (55 mg.) showed the presence of two sugars, which were separated on Whatman 3MM paper with benzene–ethanol–water as eluant to give fractions 1*a* (27 mg.) and 1*b* (28 mg.). Fraction 1*a* was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose by conversion into its aniline derivative, m. p. and mixed m. p. 144—145°, $[\alpha]_D^{17}$ $-96° \longrightarrow -41°$ (*c*, 0·1 in $COMe_2$). Fraction 1*b* was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into its aniline derivative, m. p. and mixed m. p. 195—196°, $[\alpha]_D^{16}$ $-137°$ (*c*, 0·5 in C_5H_5N).

Fraction 2. The syrup (1·640 g.) had $[\alpha]_D^{17}$ $-10°$ (*c*, 6·0 in H_2O). Chromatographic examination showed only 2 : 3 : 6-tri-*O*-methyl-D-mannose, and hypoiodite oxidation indicated 95% purity (Found: OMe, 39·8. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). The identity of the sugar was confirmed by conversion into 2 : 3 : 6-tri-*O*-methyl-*N*-phenyl-D-mannosylamine, m. p. and mixed m. p. 127—128°, $[\alpha]_D^{16}$ $-155° \longrightarrow -40°$ (*c*, 0·1 in MeOH) (Found: C, 60·9; H, 7·9; N, 4·1. Calc. for $C_{15}H_{23}O_5N$: C, 60·6; H, 7·8; N, 4·7%), 2 : 3 : 6-tri-*O*-methyl-D-mannono-γ-lactone, m. p. and mixed m. p. 80—81°, $[\alpha]_D^{20}$ $+69·5°$ (*c*, 0·6 in H_2O), and 2 : 3 : 6-tri-*O*-methyl-D-mannonic acid phenylhydrazide, m. p. and mixed m. p. 130—131°, $[\alpha]_D^{16}$ $-17°$ (*c*, 0·4 in H_2O).

Fraction 3. The syrup (66 mg.) crystallised slowly and after recrystallisation from ether had m. p. 149—151° (undepressed on admixture with fraction 4*a* of the methylated mannan A hydrolysate).

Fraction 4. The syrup (232 mg.) which partly crystallised on standing was hydrolysed with N-sulphuric acid, and chromatographic examination showed the presence of two sugars. Separation was effected on Whatman 3MM paper, butanol–ethanol–water being used as eluant to give fractions 4*a* (203 mg.) and 4*b* (29 mg.). Fraction 4*a* had m. p. 148—150° (undepressed on admixture with fraction 3). Fraction 4*b* was demethylated to give mannose and travelled on the chromatogram at the same rate as 2 : 3-di-*O*-methyl-D-mannose.

Quantitative Examination of the Methylated Sugars.—A portion of the hydrolysate of methylated mannan B was separated chromatographically and estimated by alkaline hypoiodite as described previously [Found (results expressed as c.c. of 0·01N-iodine consumed): "tetra," 0·085, 0·075; "tri," 3·50, 2·96]. These figures correspond to a molecular ratio of tetramethyl sugar : trimethyl sugar of 1 : 40.

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THE CHEMISTRY AND CHEMICAL DEGRADATION OF CELLULOSE

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Cellulose, the chief constituent of the cell walls of the majority of plants, possesses, at least to a first approximation, the same basic chemical structure from whatever source it is derived, whether from cotton where it occurs in a relatively pure state, from the underlying skeleton of wood where it occurs in close association with lignin and hemicelluloses, or as synthesized by certain micro-organisms. In all cases the cellulose molecule has been shown to consist of a long chain of 1 : 4-linked β-D-glucopyranose units.

CHEMICAL STRUCTURE

The basic chemical structure of cellulose was established by the application of the methylation technique to cellulose and to the products of partial hydrolysis. Hydrolysis of methylated cellulose yielded almost exclusively 2 : 3 : 6-trimethyl D-glucose indicating that positions 1, 4 and 5 were occupied either in ring formation or in linking adjacent glucose residues (Irvine & Hirst, 1923). The discovery that glucose normally occurs in the pyranose form (Charlton, Haworth & Peat, 1926) made it probable that the glucose residues in cellulose were linked by 1 : 4-linkages. Confirmation of the presence of pyranose rings and of the existence of 1 : 4-interunit linkages was achieved by the determination of the structure of cellobiose and cellotriose. Cellobiose, isolated in good yield as its octaacetate from the acetolysis of cellulose, was methylated to give octamethyl cellobiose, which, on hydrolysis, yielded equimolecular proportions of 2 : 3 : 6-trimethyl glucose and 2 : 3 : 4 : 6-tetramethyl glucose, thus indicating the presence of a pyranose ring in at least every other glucose residue. That a 1 : 4-linkage existed and that the second pyranose ring was present was shown by the oxidation of cellobiose to cellobionic acid, which after methylation and hydrolysis gave 2 : 3 : 4 : 6-tetramethyl glucose and the γ-lactone of 2 : 3 : 5 : 6-tetramethyl gluconic acid (Haworth, Hirst & Plant, 1927). Proof that the 1 : 4-linkage of cellobiose was the same as that of cellulose and had not arisen from a reversion product was obtained by Haworth, Hirst & Thomas (1931) who isolated octamethyl cellobiose and hendecamethyl cellotriose by the partial hydrolysis of methylated cellulose, this procedure excluding the possibility of secondary changes leading to the formation of

cellobiose and its derivatives. The β-glucosidic linkage was demonstrated by the optical rotations of cellulose degradation products and their derivatives and confirmed by the action of specific β-glucosidase enzymes, e.g. that from the snail *Helix pomatia*, on cellodextrins (Karrer & Schubert, 1926 ; Karrer, Schubert & Wehrli, 1925). That at least 99% of the glucosidic bonds in cellulose are of the same type was shown by Freudenberg & Blomqvist (1935) by polarimetric and kinetic studies of cellulose hydrolysis.

MOLECULAR SIZE

Attempts to measure the molecular size of cellulose by chemical methods have involved estimation of reducing and non-reducing end-groups. Determinations of reducing end-groups by any of the normal methods have proved applicable in the cases of degraded celluloses but not in the case of native cellulose. Determination of the molecular size of methylated cellulose by end-group assay has suffered from two main difficulties, firstly, that of obtaining complete methylation without degradation, and secondly, that of separating the small quantity of tetramethyl glucose given on hydrolysis. The presence of even small quantities of oxygen leads to severe depolymerization in the presence of the alkali used during methylation and Haworth, Hirst, Owen, Peat & Averill (1939) showed that methylation of cellulose in air gives a product of *ca.* 200 glucose residues. Recent methylation studies by McGilvray (1953) have given values of the order of 1000 for the chain length of cellulose methylated with rigorous exclusion of oxygen.

Measurement of the molecular size of cellulose and its derivatives by physical methods, e.g. osmometry, viscosity, light scattering and ultracentrifugal sedimentation has given much higher values for the degree of polymerization but these values appear to depend considerably on the method employed. The more generally accepted figure for the degree of polymerization of untreated native cellulose is in the range 3,000 to 4,000 usually obtained by viscosity measurements (Bryde & Ranby, 1947 ; Staudinger, 1937 ; Gralen, 1944). Gralen (1944) however claims values as high as 10,000 from sedimentation and diffusion measurements in cuprammonium.

CRYSTALLINE STRUCTURE

The application of X-ray diffraction studies to the structure of cellulose has supported the purely chemical evidence. Nägeli & Schwendener (1877) first observed that plant membranes were anisotropic and conceived that these membranes were built up of submicroscopic bodies which he called micelles. Early studies indicated that cellulose, from many sources, gave the same X-ray diagram. Subsequent refinements in X-ray technique revealed

periodic structure repeating itself every 10·3 A in the direction of the fibre, and on the basis of known atomic radii for carbon and oxygen the interference pattern was shown to correspond to that of the pyranose ring (Sponsler & Dore, 1926). The size of the repeating unit, which is nothing more than the length of the unit cell along the fibre axis, corresponds exactly to the length of two glucose residues (Meyer, 1937). The periodicity of such a structure in the direction of the fibre axis was explained by Mark & Meyer (1928) on the assumption of a diagonal screwing such that the two glucose residues in a cellobiose unit of cellulose are orientated at an angle of 180° to each other. Meyer & Misch (1937) represented the chains in the elementary cell of native cellulose as running in opposite directions so that the crystal is held together not only by strong covalent bonds along the fibre axis but also by hydrogen bonding between glucose residues of adjacent chains.

Cellulose molecules in native fibres were thus shown to run parallel to one another and to be arranged in a lattice, which is, however, not continuous. The ordered regions or crystallites are identical with the micelles of Nägeli and measurement of their dimensions from the breadth of the X-ray diffraction patterns by Mark & Meyer (1929), by Hess, Trogus, Akim & Sakurada (1931) and by Kratky (1944) gave values of 600–1500 A for the length in the direction of the fibre and 60–100 A for the width, these dimensions varying with the source of the cellulose. These values have been substantiated by electron microscopic measurements of the long filamentary fibrils obtained by ultrasonic treatment of cellulose fibres (Ranby & Ribi, 1950). The original micellar theory of Meyer (1930) in which the ordered regions or micelles were considered as closed entities laid parallel to one another is no longer tenable since colloidal precipitation of metals in the fibres has shown the presence of intermicellar spaces running longitudinally from one crystallite to the next (Berkmann, Böhm & Zocher, 1926). Furthermore, even the minimum values for the length of the cellulose molecule, as determined by physical measurements, are such that individual chains must pass through several crystalline regions, the disordered or amorphous regions arising where the cellulose chains are irregular. The concept of cellulose chains passing through two or more crystalline regions offers an explanation of the extremely high tensile strength of cellulose.

The chemical reactivity of cellulose in the solid state is related to the proportions of crystalline and amorphous regions. The accessibility of various parts of the fibre depends on the degree of order and the crystalline regions are penetrated by chemical reagents only with difficulty. Measurements of the relative proportions of the easily accessible amorphous regions and the difficultly accessible crystalline regions have been made physically, notably by X-ray diffraction

studies, and chemically by methods based on the preferential reactivity of the disordered regions. Hermans & Weidinger (1948, 1949) have developed X-ray measurements in which the intensity of the diffuse background of the scattered radiation was assumed to be a correct relative measure of the amorphous region. Values of *ca.* 70% crystallinity have been obtained for native cellulose fibre in cotton and of *ca.* 65% for the crystallinity of wood pulp cellulose (Hermans & Weidinger, 1949).

One of the most frequently applied chemical methods for the determination of the accessibility of cellulose is that of Nickerson & Habrle (1947) involving hydrolysis with 2·5 N-sulphuric acid followed by estimation of the glucose formed. Values of 5–10% accessibility are usually quoted for unswollen cotton and wood fibres. To account for the discrepancies between the values obtained by physical and chemical methods two opposing explanations have been put forward. Brenner, Frilette & Mark (1948) have questioned the validity of hydrolysis methods for determining crystallinity and have suggested that a recrystallization takes place during the first stages of the hydrolysis. On the other hand Ranby (1952) has recently suggested that the X-ray diffraction method does not account for the 20% of cellulose chains which lie on the surfaces of the micellar strings and which will also give rise to diffuse scattering.

CHEMICAL DEGRADATION OF CELLULOSE

Cellulose is degraded chemically by acids and by oxidizing agents, both procedures generally resulting in a more or less complete loss of fibrous structure. The generic names hydrocellulose and oxycellulose are applied to the respective products of acidic and oxidative breakdown. With dilute acids cellulose is broken down to hydrocellulose of degree of polymerization, 100–300. Haworth, Peat & Wilson (1939) obtained values of 70–200 for the chain lengths of different hydrocelluloses by the methylation procedure. Hydrolysis with 5–10% acid leads to an accumulation of short chain celluloses (100–200 residues) whereafter hydrolysis occurs only slowly. Jörgensen (1950 *a*, *b*) has shown that for cotton cellulose and sulphite pulp the exact limiting value for the degree of polymerization after hydrolysis depends on the source and previous treatment of the cellulose.

In extensive studies of the hydrolytic degradation of cellulose Ranby (1951, 1952) has described the formation of crystalline micellar particles which peptise to form aqueous solutions in the pH range 3·5–9·5 and in the absence of neutral electrolytes. These ' micelles ' are of similar size to the crystalline regions of the elementary fibrils or micellar strings observable in the electron microscope after disintegration of the cellulose fibre in aqueous suspension with ultrasonic irradiation. Electron microscopic measurements of the ' micelles

indicated widths of 80–100 A and lengths of 200–1000 A from wood and cotton cellulose, and corresponding molecular weight measurements gave values for the degree of polymerization of 50–200. Sedimentation experiments are difficult to interpret but the results indicated that the main part of the material sedimented in aggregates (Ranby, 1951). The accumulation of these 100-unit particles has usually been attributed to preferential hydrolysis of the cellulose chains in the amorphous regions. However, measurement of the crystallinity of Ranby's micelles by X-ray diffraction and of their accessibility by chemical methods gave values similar to those found for native cellulose fibres. It would appear, therefore, that the distinction between clearly defined crystalline and amorphous regions is too simple and that there must be present gradations from highly ordered to less highly ordered regions.

An alternative explanation of the hydrolytic breakdown of cellulose by an analysis of the size distribution of the hydrolysis products has led to the postulation of periodic modified linkages in the cellulose chain. Pacsu (1947) has claimed evidence for an acid-labile bond approximately every 100 glucose residues and has postulated either an acetal or hemiacetal linkage interconnecting adjacent chains. Schultz & Husemann (1946) from their studies of the acid hydrolysis and oxidative degradation of cellulose have put forward the theory that a weak bond occurs every 500 glucose residues. On the other hand recent work by Sharples (1953) has indicated that no such modified linkages are present in untreated cotton cellulose, although they appear to be present in cellulose regenerated from cuprammonium.

The oxidative breakdown of cellulose is a comparatively uncharted field and very little is known about the majority of oxidations. The term oxycellulose is applied to all oxidation products of cellulose, whatever reagent is used and whichever part of the glucose residue is attacked. The oxidative degradation of cellulose by atmospheric oxygen is greatly accelerated in the presence of alkali (Eisenhut, 1941), and, as was noted in the discussion of the methylation of cellulose, leads to severe depolymerization. Staudinger & Jurisch (1939) showed that only extremely small quantities of oxygen are required to reduce the degree of polymerization of a given cellulose by a half.

Oxidation of the primary alcoholic group to a carboxylic acid occurs with nitrogen dioxide (Kenyon & Yackel, 1942; McGee, Fowler, Taylor, Unruh & Kenyon, 1947; Unruh & Kenyon, 1942) in which case a high proportion of primary alcoholic groups are oxidized without destruction of the crystalline structure of the cellulose (Nevell, 1950). A different type of oxidation occurs with the periodate ion (Jackson & Hudson, 1938) when scission of the glycol grouping between C_2 and C_3 takes place. In this case, the formation of the polymeric aldehyde is

accompanied by a gradual destruction of the characteristic X-ray diagram (Davidson, 1941) and although no initial cleavage of glycosidic bonds occurs the product is considerably degraded by alkali. Oxidation of secondary alcoholic groups also occurs with non-specific reagents, e.g. chromic acid and hypohalites. The formation of reducing groups is indicated by high copper numbers but here again degradation involving cleavage of the cellulose chain appears to be due to the alkali sensitivity of the carbonyl groups. Little is known about the mechanism of formation of such reducing oxycelluloses although Davidson (1941) has shown that the products are different in character from those formed by the action of periodic acid. The initially highly reducing non-acidic oxycelluloses on further oxidation develop acidic properties, the acidic character being derived partially from oxidation of carbon atoms 2 and/or 3 and partially from oxidation of C_6 to give uronic acid residues (Davidson & Nevell, 1947).

The results of the chemical degradation of cellulose have thrown some light on the structure of the cellulose fibre but the fact that the majority of such reactions are heterogeneous makes their kinetic interpretation difficult. Although in some hydrolytic reactions the results have been explained in terms of modified linkages, they may be at least equally well explained in terms of the differential accessibility of the various parts of the fibre. Because of the ease with which oxidation occurs in the presence of alkali the results of reactions of pre-treated cellulose should be interpreted with caution. Certainly, no purely chemical evidence for the presence of modified linkages in native cellulose has yet been brought forward.

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THE METHYL ETHERS OF D-MANNOSE

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Contents

I. Introduction

The importance of the methyl ethers of D-mannose lies largely in their occurrence in structural studies in the polysaccharide field. Since previous articles in this series[1,2,3] have discussed the basic principles involved in the preparation and elucidation of the structure of partially methylated aldoses, it is not intended to give unnecessary details in cases where general procedures are already familiar.

In every case so far investigated, D-mannose has been found to occur

(1) E. J. Bourne and S. Peat, *Advances in Carbohydrate Chem.*, **5**, 145 (1950).
(2) D. J. Bell, *Advances in Carbohydrate Chem.*, **6**, 11 (1951).
(3) R. A. Laidlaw and E. G. V. Percival, *Advances in Carbohydrate Chem.*, **7**, 1 (1952).

naturally in the pyranose form, and apart from 2,3,5-trimethyl-D-mannofuranose and 2,3,5,6-tetramethyl-D-mannofuranose, no methylated derivatives containing a furanose ring have so far been synthesized. In the preparation of partially methylated derivatives, use has been made of benzylidene, isopropylidene and trityl derivatives of D-mannose to protect respectively the 4 and 6, 2 and 3, and 6 positions.

II. Monomethyl-D-mannoses

1. *2-Methyl*-D-*mannose*

2-Methyl-D-mannose was first obtained crystalline by Pacsu and Trister.[4] Methylation of 3,4:5,6-diisopropylidene-D-mannose dibenzyl mercaptal by treatment of the sodium alkoxide with methyl iodide was followed by removal of the isopropylidene residues, giving 2-methyl-D-mannose dibenzyl mercaptal. The mercaptal group was removed in dry methanol with mercuric chloride, giving a mixture of the methyl α-D-furanoside and the dimethyl acetal. From the hydrolysis of the methyl α-D-furanoside, crystalline 2-methyl-α-D-mannose was isolated. It is probable that this sugar had been obtained before in an impure state, Pacsu and v. Kary[5] having previously methylated diisopropylidene-D-mannose dibenzyl mercaptal and having obtained a sirup which they wrongly supposed to be the 4-methyl ether. Subsequent work by Munro and Percival[6] showed that Pacsu and v. Kary's product was not the 4-methyl ether, but these workers were unable to isolate either the 2-methyl ether or any of its derivatives in a pure state.

Very little conclusive evidence for the position of the methyl group in 2-methyl-D-mannose is available. Pacsu and Trister[4] showed that the sugar reacted with phenylhydrazine in the cold to give a phenylhydrazone but not a phenylosazone. More drastic conditions were required to form the phenylosazone, during which reaction the methyl group was lost with the formation of D-glucosazone.

2. *4-Methyl*-D-*mannose*

The synthesis of 4-methyl-D-mannose was first achieved simultaneously, but independently, in two different ways. Haskins, Hann and Hudson[7] methylated 2,3-isopropylidene-1,6-anhydro-β-D-mannopyranose with methyl iodide and silver oxide, the product on hydrolysis yielding

(4) E. Pacsu and S. M. Trister, *J. Am. Chem. Soc.*, **63**, 925 (1941).

(5) E. Pacsu and Charlotte v. Kary, *Ber.*, **62**, 2811 (1929).

(6) J. Munro and E. G. V. Percival, *J. Chem. Soc.*, 640 (1936).

(7) W. T. Haskins, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 7 (1943).

the crystalline 4-methyl-α-D-mannose. Schmidt and his coworkers[8,9] carried out an alternative synthesis by methylation of 2,3:5,6-diisopropylidene-D-mannonic acid with dimethyl sulfate and sodium hydroxide, followed by removal of the isopropylidene residues to give 4-methyl-D-mannonic acid, isolated as the δ-lactone. Catalytic reduction of the lactone over platinic oxide gave a mixture of the free sugar and the corresponding hexitol, the sugar being isolated via the benzylphenylhydrazone. The sugar has also been synthesized by Smith,[10] who methylated methyl 2,3-isopropylidene-6-trityl-α-D-mannopyranoside, 4-methyl-D-mannose being obtained as a viscous syrup after methanolysis and hydrolysis.

The structure of this sugar was established as follows: (a) reaction with phenylhydrazine yielded 4-methyl-D-glucosazone;[11] (b) oxidation (HOBr) yielded a methylmannonolactone, which exhibited the properties that are characteristic of δ-lactones.[7]

3. *6-Methyl-D-mannose*

Watters, Hockett and Hudson[12] have prepared a non-crystalline monomethylmannose which forms an osazone with the same properties as those of 6-methyl-D-glucosazone. The synthesis was achieved by methylation of methyl 2,3,4-triacetyl-α-D-mannopyranoside, followed by hydrolysis. Schmidt and Heiss,[13] studying the epimerization of 6-methyl-D-gluconic acid, have claimed to have isolated the phenylhydrazide of 6-methyl-D-mannonic acid.

4. *Characterization of Monomethyl-D-mannoses*

Table I records appropriate data and references relating to the monomethyl-D-mannoses and their more important derivatives.

(8) O. T. Schmidt, Catharina C. Weber-Molster and Helen Hauss, *Ber.*, **76**, 339 (1943).

(9) O. T. Schmidt and Hertha Müller, *Ber.*, **76**, 344 (1943).

(10) F. Smith, *J. Chem. Soc.*, 2652 (1951).

(11) A. E. Knauf, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **63**, 1447 (1941).

(12) A. J. Watters, R. C. Hockett and C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 1528 (1939).

(13) O. T. Schmidt and H. Heiss, *Ber.*, **82**, 7 (1949).

TABLE I
Monomethyl-D-mannoses and Some of Their Characteristic Derivatives

Compound	*Melting point, °C.*	$[\alpha]_D$, °	*Rotation solvent*	*References*
2-Methyl-α-D-mannose	136–137	+7.0 → +4.5	H_2O	4
phenylhydrazone	163	−49.1 → −60.7	C_5H_5N	4
dimethyl acetal	111–112	−11.3	H_2O	4
methyl α-D-furanoside	82	+129.5	H_2O	4
4-Methyl-α-D-mannose	127–128	+34.0 → +22.6	H_2O	7
	129–130	+32.4(±0.5) → +22.3	H_2O	9
phenylosazone	158	−32.3(±2.5) → 0	C_2H_5OH	9
	157–158	−36 → −14.4	C_5H_5N	7
benzylphenylhydrazone	128–130	+49.2(±0.6) → +46.9	CH_3OH	9
α-tetraacetate	75–76	+59.2	$CHCl_3$	7
(?)β-tetraacetate	63–64	+20.2	$CHCl_3$	7
methyl α-D-pyranoside	101–103	+84.9(±0.9)	H_2O	9
	101–102	+83.9	H_2O	7
4-Methyl-D-mannonic acid	—	+24(±0.4)	H_2O	8
sodium salt	—	+2(±0.4)	H_2O	8
δ-lactone	164–165	+162.3 → +94.3	H_2O	8
	165–166	+163.8 → +94.2	H_2O	7
phenylhydrazide	147–148	+10	H_2O	8
	146–147	+10.6	H_2O	7
amide	176	+11.7	H_2O	8
	171–172	+11.9	H_2O	7
6-Methyl-D-mannose	—	+15.3	$CHCl_3$	12
phenylosazone	172	−68.6 → −48.0	C_2H_5OH	12
6-Methyl-D-mannonic acid phenylhydrazide	178	+3.5(±0.7)	H_2O	13

III. DIMETHYL-D-MANNOSES

1. *2,3-Dimethyl-D-mannose*

2,3-Dimethyl-D-mannose has been isolated from the hydrolysis products of the methylated galactomannans from carob seed gum[14,15] and from guar gum,[16,17] and also from mannocarolose,[18,19] a polysaccharide synthesized by *Penicillium charlesii* G. Smith. The synthesis of this sugar, which has not been obtained crystalline, has been accom-

(14) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1278 (1948).
(15) F. Smith, *J. Am. Chem. Soc.*, **70**, 3249 (1948).
(16) Z. F. Ahmed and R. L. Whistler, *J. Am. Chem. Soc.*, **72**, 2524 (1950).
(17) C. M. Rafique and F. Smith, *J. Am. Chem. Soc.*, **72**, 4634 (1950).
(18) W. N. Haworth, H. Raistrick and M. Stacey, *Biochem. J.*, **29**, 612 (1935).
(19) M. Stacey, *J. Chem. Soc.*, 857 (1947).

plished by Robertson.[20] Methyl 4,6-benzylidene-α-D-mannopyranoside was methylated with methyl iodide and silver oxide, the benzylidene residue removed with hot aqueous acetonic hydrogen chloride and the glycoside hydrolyzed to yield sirupy 2,3-dimethyl-D-mannose.

The constitution of the sugar rests upon the following evidence: (a) reaction with phenylhydrazine eliminated a methyl group with the formation of 3-methyl-D-glucosazone;[20] (b) oxidation of the derived lactone[21] and of the methyl glycoside[17] with nitric acid gave rise to *erythro*-dimethoxysuccinic acid; (c) periodate oxidation consumed 2 moles of periodate per mole of dimethyl sugar with the production of both formic acid and formaldehyde.[17]

2. *3,4-Dimethyl-D-mannose*

Although this sugar has not yet been synthesized, it has been isolated from the products of methylation and hydrolysis of yeast mannan,[22,23] the galactomannan from Lucerne seed,[24] and the specific somatic[25] and lipoid-bound[26] polysaccharides from *M. tuberculosis* (Human Strain). The constitution of 3,4-dimethyl-D-mannose was established by Haworth, Hirst and Isherwood.[22] The presence of a methyl group in position 4 was indicated since no furanoside formation took place with cold methanolic hydrogen chloride, and oxidation (HOBr) yielded a crystalline dimethyl-D-mannonolactone, which from its rate of hydrolysis was shown to belong to the δ-series. 4,6-Dimethyl-D-mannose being already known, the choice between the 2,4- and 3,4-dimethyl sugars was easily made as the sugar formed an osazone without loss of a methyl group, and the amide derived from the dimethylmannonolactone gave a strong positive Weerman test. Later work[27] showed that periodate oxidation gave a definite though not quantitative yield of formaldehyde, thus confirming the absence of a methyl group in position 6.

3. *4,6-Dimethyl-D-mannose*

This sugar has been synthesized by Ault, Haworth and Hirst.[28] Methyl 2,3-isopropylidene-α-D-mannopyranoside was methylated with

(20) G. J. Robertson, *J. Chem. Soc.*, 330 (1934).
(21) E. H. Goodyear and W. N. Haworth, *J. Chem. Soc.*, 3136 (1927).
(22) W. N. Haworth, E. L. Hirst and F. A. Isherwood, *J. Chem. Soc.*, 784 (1937).
(23) W. N. Haworth, R. L. Heath and S. Peat, *J. Chem. Soc.*, 833 (1941).
(24) E. L. Hirst, J. K. N. Jones and Winifred O. Walder, *J. Chem. Soc.*, 1443 (1947).
(25) W. N. Haworth, P. W. Kent and M. Stacey, *J. Chem. Soc.*, 1211 (1948).
(26) W. N. Haworth, P. W. Kent and M. Stacey, *J. Chem. Soc.*, 1222 (1948).
(27) D. J. Bell, *J. Chem. Soc.*, 992 (1948).
(28) R. G. Ault, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1012 (1935).

methyl iodide and silver oxide, and the isopropylidene residue removed by mild acid hydrolysis to give methyl 4,6-dimethyl-α-D-mannopyranoside, which on more vigorous hydrolysis yielded 4,6-dimethyl-D-mannose as a glass. The constitution of the sugar was established as follows: (a) oxidation (HOBr) gave a dimethylmannonolactone which behaved exclusively as a δ-lactone; (b) the derived dimethylmannonamide gave a positive Weerman test indicating a free hydroxyl in position 2; (c) oxidation of methyl 2,3-isopropylidene-α-D-mannoside,[29] followed by elimination of the isopropylidene residue, gave methyl α-D-mannuronoside, indicating a free hydroxyl group in position 6 in the starting material and hence a methyl group after methylation.

4. *5,6-Dimethyl-D-mannose*

Although 5,6-dimethyl-D-mannose itself is unknown, some of its derivatives have been prepared. Irvine and Patterson[30] prepared a dimethylmannitol by the methylation of 1,2:3,4-diisopropylidene-D-mannitol followed by hydrolysis of the isopropylidene residues with aqueous ethanolic hydrogen chloride. Nitric acid oxidation of the dimethylmannitol gave a dimethylmannonolactone with behavior characteristic of a γ-lactone. The structure of the diisopropylidene-mannitol, however, was not proved until Wiggins[31] obtained a strongly reducing diisopropylidene-*aldehydo*-D-arabinose on oxidation with lead tetraacetate, showing the isopropylidene residues to be attached at positions 1, 2, 3, and 4.

5. *Characterization of Dimethyl-D-mannoses*

Table II records appropriate data and references relating to the dimethyl-D-mannoses and their more important derivatives.

(29) R. G. Ault, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 517 (1935).
(30) J. C. Irvine and Bina M. Patterson, *J. Chem. Soc.*, **105**, 898 (1914).
(31) L. F. Wiggins, *J. Chem. Soc.*, 13 (1946).

TABLE II

Dimethyl-D-mannoses and Some of Their Characteristic Derivatives

Compound	*Melting point, °C.*	$[\alpha]_D$, °	*Rotation solvent*	*References*
2,3-Dimethyl-D-mannose	liquid	+6.0	CH_3OH	20
		+10.6	C_2H_5OH	20
		−4.3	$CHCl_3$	20
		−15.8	H_2O	20
oxime	112–114	—	—	20
methyl α-D-pyranoside	liquid	+43.5	$CHCl_3$	20
2,3-Dimethyl-D-mannonic acid	—	−31.0 → 0 (incomplete)	H_2O	15
γ-lactone	109–110	+61.1 → +60.5 (incomplete)	H_2O	21
	107	+60 → +57 (incomplete)	H_2O	14
	111	+61.5 → +52.5 (incomplete)	H_2O	15
		+64.5 → +35 (incomplete)	H_2O	17
phenylhydrazide	170	—	—	14
	158(168)	−25	H_2O	15
	156	−24.2	H_2O	17
3,4-Dimethyl-D-mannose	107–109	+3 (equil.)	H_2O	22
α-monohydrate	114	+30.0	CH_3OH	23
		+22 → +4	H_2O	23
1,2-isopropylidene-	94	−17	H_2O	23
methyl α-D-pyranoside	87	+67	H_2O	23
		+107	$CHCl_3$	23
3,4-Dimethyl-D-mannonic acid	—	+32	H_2O	22
δ-lactone	157–158	+174 → +129 (50 hr., equil.)	H_2O	22
	159–160	+178 → +131 (120 hr., equil.)	H_2O	23
amide	140	+22	H_2O	22
	141	+25.7	H_2O	23
4,6-Dimethyl-D-mannose	glass	+25	H_2O	28
2,3-isopropylidene-α-D-pyranose	76–77	+11	CH_3OH	28
		0 → −9.5 (6 hr., equil.)	H_2O	28
methyl α-D-pyranoside	liquid	+80.5	H_2O	28
		+99	CH_3OH	28
4,6-Dimethyl-D-mannonic acid	—	+20 → +68 (150 hr., equil.)	H_2O	28
δ-lactone	55	+165 → +70 (150 hr., equil.)	H_2O	28
		+145	C_2H_5OH	28
amide	119	−3	CH_3OH	28
		+15	H_2O	28
phenylhydrazide	151	−3.5	C_2H_5OH	28
		+14	H_2O	28
5,6-Dimethyl-D-mannonic acid γ-lactone	112–114	+22.4 → +16.2 (6 days, equil.)	CH_3OH	30

IV. Trimethyl-D-mannoses

1. *2,3,4-Trimethyl-D-mannose*

The first synthesis of this sugar, which is known only as a sirup, was achieved by Haworth, Hirst, Isherwood and Jones[32] by the methylation of methyl 6-trityl-α-D-mannopyranoside. The methyl 6-trityl-2,3,4-trimethyl-α-D-mannoside thus obtained was treated with hot glacial acetic acid to remove the trityl residue, and the resulting glycoside gave on hydrolysis 2,3,4-trimethyl-D-mannose. The sugar has also been synthesized by the methylation of 1,6-anhydro-β-D-mannopyranose[33] with dimethyl sulfate and alkali, followed by hydrolytic cleavage of the anhydro ring with dilute acid. 2,3,4-Trimethyl-D-mannose has been shown to be present amongst the hydrolysis products of methylated yeast mannan,[23] having been isolated as the derived δ-lactone. The crystalline trimethylmannose isolated from the hydrolysis of methylated mannocarolose,[18] and described as the 2,3,4-compound, differed markedly in its properties from the synthetic compound and was obviously a different isomer. Recent work,[19] however, has shown that 2,3,4-trimethyl-D-mannose and the crystalline 3,4,6-isomer occur together in equimolecular proportions in the hydrolyzate of methylated mannocarolose.

The constitution of the sugar follows from the methods employed in its synthesis. Confirmation was obtained by the following observations: (a) permanganate oxidation of the methyl α-D-glycopyranoside yielded 2,3,4-trimethyl-D-mannuronic acid[34] on hydrolysis of the resulting glycoside; (b) oxidation (HOBr) gave a characteristic δ-lactone.

2. *2,3,5-Trimethyl-D-mannose*

This sugar was prepared by Heslop and Smith[35] by methylation of methyl 6-trityl-α-D-mannofuranoside with methyl iodide and silver oxide, followed by removal of the trityl residue with ethereal hydrogen chloride and hydrolysis of the glycoside to yield 2,3,5-trimethyl-D-mannose as a sirup. The constitution of the sugar follows largely from the method of synthesis. Confirmation was obtained by the following observations: (a) oxidation (HOBr) yielded a lactone with properties characteristic of γ-lactones; (b) further oxidation gave a trimethylmannosaccharic acid whose diamide gave a negative Weerman test.

(32) W. N. Haworth, E. L. Hirst, F. A. Isherwood and J. K. N. Jones, *J. Chem. Soc.*, 1878 (1939).

(33) G. Zemplén, A. Gerecs and Theodora Valatin, *Ber.*, **73**, 575 (1940).

(34) F. Smith, M. Stacey and P. I. Wilson, *J. Chem. Soc.*, 131 (1944).

(35) (Miss) D. Heslop and F. Smith, *J. Chem. Soc.*, 574 (1944).

3. *2,3,6-Trimethyl-D-mannose*

Although this sugar has not been prepared synthetically, it is a constituent of the hydrolysis products of methylated polysaccharides containing mannose units linked 1 to 4, for example, the mannans from ivory nut,[36–38] salep,[39] and the seaweed *Porphyra umbilicalis*,[40] and from the galactomannans from carob[14,15] and guar[16,17] seeds. The structure of the sugar follows from the work of Haworth, Hirst and Streight.[41] Methylation with methyl iodide and silver oxide gave a mixture of methyl 2,3,4,6-tetramethyl-D-mannopyranosides from which the methyl β-D-glycoside was obtained crystalline and from the hydrolysis of which the 2,3,4,6-tetramethyl-D-mannose was characterized as the anilide. The presence of a second free hydroxyl group, in position 4, was shown by oxidation (HOBr) of the free sugar to a γ-lactone which on methylation gave 2,3,5,6-tetramethyl-D-mannonolactone.

4. *2,4,6-Trimethyl-D-mannose*

This sugar was isolated as its monohydrate from the hydrolysis products of methylated yeast mannan and its structure proved by Haworth, Heath and Peat.[23] Methylation of the methyl glycoside gave the crystalline methyl 2,3,4,6-tetramethyl-α-D-mannopyranoside, indicating a pyranose structure. It seemed likely that the compound was the monohydrate of 2,4,6-trimethyl-D-mannose, as the 2,3,4- and 2,3,6-isomers were known as sirups that did not form hydrates, and the crystalline 3,4,6-isomer could not be converted into a hydrate; furthermore, the derived anilide was different from those of the 2,3,6- and 3,4,6-trimethyl-D-mannoses. Oxidation (HOBr) gave a δ-lactone that is different from the isomeric 2,3,4- and 3,4,6-trimethyl-D-mannonolactones, and the derived amide gave a negative Weerman test, confirming the presence of a methyl group in position 2.

5. *3,4,6-Trimethyl-D-mannose*

3,4,6-Trimethyl-D-mannose was first synthesized by Bott, Haworth and Hirst,[42] during their study of the obstructed form of methyl 2-acetyl-α-D-mannoside, methylation of which compound, followed by acid

(36) J. Patterson, *J. Chem. Soc.*, **123**, 1139 (1923).
(37) F. Klages, *Ann.*, **509**, 159 (1934).
(38) F. Klages, *Ann.*, **512**, 185 (1934).
(39) F. Klages and R. Niemann, *Ann.*, **523**, 224 (1936).
(40) J. K. N. Jones, *J. Chem. Soc.*, 3292 (1950).
(41) W. N. Haworth, E. L. Hirst and H. R. L. Streight, *J. Chem. Soc.*, 1349 (1931).
(42) H. G. Bott, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1395 (1930).

hydrolysis, yielded the sugar in its crystalline α-pyranose form. The sugar has also been derived from the hydrolysis of methylated polysaccharides, notably from yeast mannan,[23] the carbohydrate residue in ovomucoid,[43] and the specific somatic polysaccharide from *M. tuberculosis*,[25] and also from the methylation and hydrolysis of the aldobiouronic acid obtained from damson[44] and cherry gums.[45]

The constitution of this sugar was established as follows: (a) oxidation (HOBr) gave a δ-lactone having the same melting point and the same magnitude of rotation, but of opposite sign, as 3,4,6-trimethyl-L-mannonolactone synthesized by Haworth and Peat[46] from 2,3,5-trimethyl-L-arabofuranose; (b) further methylation[42] yielded the known methyl 2,3,4,6-tetramethyl-β-D-mannopyranoside, thus confirming the presence of a pyranose ring; (c) treatment of the δ-lactone with liquid ammonia produced an amide[44] giving a positive Weerman test, thus showing the presence of a free hydroxyl in position 2.

6. *Characterization of Trimethyl-D-mannoses*

Table III records appropriate data and references relating to the trimethyl-D-mannoses and their more important derivatives.

(43) M. Stacey and J. M. Woolley, *J. Chem. Soc.*, 550 (1942).
(44) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1174 (1938).
(45) J. K. N. Jones, *J. Chem. Soc.*, 558 (1939).
(46) W. N. Haworth and S. Peat, *J. Chem. Soc.*, 350 (1929).

TABLE III

Trimethyl-D-mannoses and Some of Their Characteristic Derivatives

Compound	*Melting point, °C.*	$[\alpha]_D$, °	*Rotation solvent*	*References*
2,3,4-Trimethyl-D-mannose	liquid	+2	H_2O	32
		−5	H_2O	33
1,6-anhydro	52	−70.7	$(CH_3)_2CO$	33
		−65.5	H_2O	33
methyl α-D-pyranoside	liquid	+47	H_2O	34
2,3,4-Trimethyl-D-mannonic acid				
δ-lactone monohydrate	73	+138 → +81 (95 hr., equil.)	H_2O	32
	74	+131 → +80 (170 hr., equil.)	H_2O	34
	72–73	+129.5 → +78.4 (72 hr., equil.)	H_2O	33
amide	143	+5	H_2O	32, 34
phenylhydrazide	166	—	—	40
2,3,5-Trimethyl-D-mannonic acid	—	−31	H_2O	35
sodium salt	—	−27	H_2O	35
γ-lactone	118	+67 → +63.5 (22 days, incomplete)	H_2O	35
amide	162	−28	H_2O	35
2,3,6-Trimethyl-D-mannose	liquid	+6	H_2O	47
		−10	H_2O	14
anilide	127–128	—	—	41, 47
	133	—	—	14
	131	−155 → −39	CH_3OH	15
2,3,6-Trimethyl-D-mannonic acid		−19.5 → +39	H_2O	15
	—	(23 days)		
γ-lactone	89	+73 → +67 (6 days, incomplete)	H_2O	41
	84–85	+65.5 → +38.5 (120 days)	H_2O	15
amide	130	—	—	14
	125	−21	H_2O	15, 16
phenylhydrazide (anhydrous)	144	−16.5	H_2O	17
phenylhydrazide hydrate	133	−21	H_2O	15, 17
2,4,6-Trimethyl-D-mannose				
α-pyranose monohydrate	90	+21 → +14 (2 hr.)	H_2O	23
β-pyranose	104–107	−5.7 → +19.0	H_2O	23
anilide	134	−150 → +8 (13 hr.)	CH_3OH	23

TABLE III (*Continued*)

Compound	*Melting point, °C.*	$[\alpha]_D$, °	*Rotation solvent*	*References*
2,4,6-Trimethyl-D-mannonic acid				
δ-lactone	97–98	+141 → +30 (103 hr. at 30°)	H_2O	23
amide	145	+7.0	H_2O	23
3,4,6-Trimethyl-α-D-mannose	101–102	+36	CH_3OH	42
		+21 → +8.2	H_2O	42
	104	—	—	43
anilide	140–143	+154.5 → −55.5 (24 hr.)	CH_3OH	23
3,4,6-Trimethyl-D-mannonic acid	—	+31 → +111	H_2O	42
δ-lactone	96–97	+167.5 → +110 (74 hr., equil.)	H_2O	42
	99–100	+168 → +116	H_2O	44
phenylhydrazide	137–139	—	—	42
amide	141	+25	H_2O	44
	143	+28	H_2O	23

V. TETRAMETHYL-D-MANNOSES

1. *2,3,4,6-Tetramethyl-D-mannose*

This sugar was first prepared by Irvine and Moodie[48] by the methyla tion of methyl α-D-mannopyranoside with methyl iodide and silver oxid in the presence of methanol as solvent. The crystalline methyl tetra methyl-α-D-mannoside yielded, on acid hydrolysis, sirupy 2,3,4,6-tetra methyl-D-mannose. Methylation has been carried out subsequentl using dimethyl sulfate and sodium hydroxide[49,50] and by the reaction o the potassium salt of methyl α-D-mannopyranoside with methyl iodid in liquid ammonia.[51] The Haworth methylation procedure has also bee

(47) W. N. Haworth, E. L. Hirst and Millicent M. T. Plant, *J. Chem. Soc.*, 19 (1931).

(48) J. C. Irvine and Agnes M. Moodie, *J. Chem. Soc.*, **87**, 1462 (1905).

(49) W. N. Haworth, *J. Chem. Soc.*, **107**, 8 (1915).

(50) H. D. K. Drew, E. H. Goodyear and W. N. Haworth, *J. Chem. Soc.*, 19 1237 (1927).

(51) I. E. Muskat, *J. Am. Chem. Soc.*, **56**, 693 (1934).

extended to the β-D-series.[52] The sugar was first obtained crystalline by Greene and Lewis.[53]

2,3,4,6-Tetramethyl-D-mannose has been obtained from the hydrolysis products of a number of methylated polysaccharides, for example the mannans from ivory nut,[37,38] salep,[39] yeast[22,23] and from the seaweed *Porphyra umbilicalis*.[40] The structure of the sugar was confirmed by the following observations: (a) oxidation (HOBr) gave a lactone that mutarotated as a δ-lactone:[50] (b) further oxidation with nitric acid yielded D-*arabo*-trimethoxyglutaric acid, identified as the crystalline methylamide.[21]

2. *2,3,5,6-Tetramethyl-D-mannose*

2,3,5,6-Tetramethyl-D-mannose was first prepared by Irvine and Burt[54] by the methylation of methyl "γ"-D-mannoside (now known to be a mixture of the methyl α- and β-D-mannofuranosides) with methyl iodide and silver oxide, followed by hydrolysis to the free sugar. Later work,[55] however, threw doubt upon the purity of the tetramethyl-D-mannose thus obtained. Haworth, Hirst and Webb, starting with crystalline methyl α-D-mannofuranoside, carried out the methylation both with dimethyl sulfate and sodium hydroxide and with methyl iodide and silver oxide, and obtained crystalline methyl tetramethyl-α-D-mannofuranoside, which was readily hydrolyzed with dilute mineral acid to yield the sirupy 2,3,5,6-tetramethyl-D-mannose. The constitution of this sugar follows from the fact that oxidation (HOBr) gave the crystalline 2,3,5,6-tetramethyl-D-mannonolactone,[56] which had previously been prepared by the methylation of γ-D-mannonolactone.[57] This methylated lactone mutarotated as a γ-lactone and further oxidation with nitric acid gave only *erythro*-dimethoxy-succinic acid and no D-*arabo*-trimethoxyglutaric acid.[21]

3. *Characterization of Tetramethyl-D-mannoses*

Table IV records appropriate data and references relating to the tetramethyl-D-mannoses and their more important derivatives.

(52) H. G. Bott, W. N. Haworth, E. L. Hirst and R. S. Tipson, *J. Chem. Soc.*, 2653 (1930).

(53) R. D. Greene and W. L. Lewis, *Science*, **64**, 206 (1926); *J. Am. Chem. Soc.*, **50**, 2813 (1928).

(54) J. C. Irvine and W. Burt, *J. Chem. Soc.*, **125**, 1343 (1924).

(55) W. N. Haworth, E. L. Hirst and J. I. Webb, *J. Chem. Soc.*, 651 (1930).

(56) P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, **76**, 809 (1928).

(57) P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, **60**, 167 (1924).

TABLE IV

Tetramethyl-D-mannoses and Some of Their Characteristic Derivatives

Compound	*Melting point, °C.*	$[\alpha]_D$, °	*Rotation solvent*	*References*
2,3,4,6-Tetramethyl-D-mannose	liquid	+1.2	H_2O	48
	50.5–51.5	+27.6	CH_3OH	53
		+23.0	$CHCl_3$	53
		+2.4 (equil.)	H_2O	53
α-pyranose form	49–50	+11.5 → +2.5	H_2O	58
anilide	142–143	−87.9 → −8.3	CH_3OH	59
		−95.5 → −38.9	$(CH_3)_2CO$	59
	144–145	−84.0 → −7.5 (11 hr.)	CH_3OH	23
methyl α-D-pyranoside	37–38	+42.9	H_2O	48
		+70.5	CH_3OH	48
		+75.5	C_2H_5OH	48
	38–40	+43	H_2O	52
methyl β-D-pyranoside	36–37	−80	H_2O	52
		−79	CH_3OH	52
		−82	C_2H_5OH	52
		−87	$CHCl_3$	52
		−72	C_6H_6	52
2,3,4,6-Tetramethyl-D-mannonic acid	—	+14.8 (initially)	H_2O	50
	—	+17.5 → +42.0 (24 hr., incomplete)	H_2O	57
sodium salt	—	+41.6	H_2O	57
δ-lactone	24–25	+150 (initially)	H_2O	50
	liquid	+136.4 → +62.8 (6 days)	H_2O	53
	25	+150 → +67 (100 hr., equil.)	H_2O	22
phenylhydrazide	184–185	—	—	50
	186–187	—	—	28
	183–184	−22	$CHCl_3$	46
2,3,5,6-Tetramethyl-D-mannose	liquid	+47.4	C_2H_5OH	54
		+48.5	CH_3OH	54
		+39 → +43	H_2O	55
		+37	CH_3OH	55
methyl α-D-furanoside	24	+99	H_2O	55
2 3,5,6-Tetramethyl-D-mannonic acid	—	−25.3 → +48.2	H_2O	57
sodium salt	—	−22.5	H_2O	57
γ-lactone	107–108	+64.8	H_2O	56
	107	+65.2 → +56.3 (incomplete)	H_2O	57
	108–109	—	—	41
phenylhydrazide	167	—	—	21

(58) B. C. Hendricks and R. E. Rundle, *J. Am. Chem. Soc.*, **60**, 2563 (1938).

(59) J. C. Irvine and D. McNicoll, *J. Chem. Soc.*, **97**, 1449 (1910).

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The Constitution of a Wheat-straw Xylan.

By G. O. Aspinall and R. S. Mahomed.

[Reprint Order No. 5047.]

Fractionation of wheat-straw hemicellulose yielded a xylan, devoid of arabinose residues, but containing uronic acid residues (*ca.* 3%). Hydrolysis of the methylated polysaccharide gave 2 : 3 : 4-tri-*O*-methyl-D-xylose (2·4%), 2 : 3-di-*O*-methyl-D-xylose (93%) and 2-*O*-methyl-D-xylose (3·4%), together with some 2-*O*-methyl-3-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose. It is concluded that this xylan has a straight chain of 40—45 D-xylopyranose residues with a single D-glucopyranuronic acid residue linked as a side-chain through position 3.

The lignified tissues of grasses and straws yield, on alkaline extraction after delignification, hemicelluloses containing D-xylose residues together with 5—10% of L-arabinose residues. In some hemicelluloses D-glucuronic acid residues are also present. Recent work in this laboratory has shown that at least two molecular types are present in esparto grass: a xylan, devoid of arabinose residues, consisting of a singly branched chain of 75 (±5) D-xylopyranose units (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289); and an araboxylan in which at least the majority of L-arabinose residues are present as side-chains attached to a main chain of 1 : 4-linked D-xylopyranose residues (Aspinall, Hirst, Moody, and Percival, *J.*, 1953, 1631). Another xylan containing no arabinose residues has been isolated from the cell-wall of ripe pears. This polysaccharide was shown to contain 115 (±5) D-xylopyranose units in a singly branched chain but in addition to carry a terminal D-glucopyranuronic acid unit at one point (Chanda, Hirst, and Percival, *J.*, 1951, 1240). The present investigation was undertaken to determine the structure of the xylan from wheat straw, a material known to be rich in pentosan.

Wheat-straw hemicellulose was extracted from the delignified straw with cold aqueous sodium hydroxide. Hydrolysis of the polysaccharide indicated the presence of arabinose (6·2%) in addition to xylose residues. After repeated precipitations, as the copper complex, with Fehling's solution, had failed to give a xylan devoid of arabinose residues, the arabinose-rich fraction was removed by extraction of the hemicellulose with hot 70% aqueous alcohol, leaving behind a xylan which gave only a trace of arabinose (*ca.* 0·5%) on hydrolysis.

The xylan was methylated under nitrogen with sodium hydroxide and methyl sulphate, and subsequently with methyl iodide and silver oxide, to give a product which was fractionated in boiling chloroform–light petroleum to give a methylated xylan. This was hydrolysed successively with methanolic and with aqueous hydrochloric acid, and the resulting sugars were separated on cellulose. The following sugars were isolated and characterised as crystalline derivatives: (1) 2 : 3 : 4-tri-*O*-methyl-D-xylose (2·4%); (2) 2 : 3-di-*O*-methyl-D-xylose (93%); and (3) 2-*O*-methyl-D-xylose (3·4%). In addition a tetramethyl aldobiuronic acid (*ca.* 1%) was isolated, which after reduction with lithium aluminium hydride (Lythgoe and Trippett, *J.*, 1950, 1983) followed by hydrolysis and chromatographic separation gave 2-*O*-methylxylose and 2 : 3 : 4-tri-*O*-methylglucose. The tetramethylaldobiuronic acid, which had a high rotation ($[\alpha]_D^{20}$ +97·5°) suggestive of an α-glycosidic linkage, was attacked by sodium metaperiodate (consumption, 0·8 mol. of periodate in 26 hr.) but no formic acid was produced. It seems probable, therefore, that the aldobiuronic acid was the same as that isolated from methylated pear cell-wall xylan (Chanda, Hirst, and Percival, *loc. cit.*), namely, 2-*O*-methyl-3-*O*-(2 : 3 : 4-tri-*O*-methyl-D-

glucopyruronosyl)-α-D-xylose. Quantitative chromatography indicated that tri-, di-, and mono-methylxylose were present in the ratio of 1 : 34 : 1.

Vigorous hydrolysis of the wheat-straw xylan gave only one acidic substance, which travelled on the chromatogram at the same rate as glucuronic acid. Under similar conditions of hydrolysis (see Aspinall, Hirst, and Mahomed, succeeding paper) residues of 4-*O*-methyl-D-glucuronic acid are partly demethylated to D-glucuronic acid but the methylglucuronic acid may still be detected chromatographically. It is probable therefore that only D-glucuronic acid residues are present in the xylan. Furthermore the low methoxyl content of the polysaccharide suggested that few, if any, uronic acid residues were present as the methyl ether. The discrepancy between the determined uronic anhydride (3·2%) in the xylan and the amount of tetramethylaldobiuronic acid (mol. % *ca.* 1) isolated from the hydrolysis of the methylated xylan may arise from decomposition of uronic acid residues during hydrolysis or from loss of the acidic components during deionisation of the hydrolysate.

A molecular-weight determination by the isothermal-distillation method (by the courtesy of Dr. C. T. Greenwood) gave a value of 8000 ± 500 (degree of polymerisation 47—53) for the methylated xylan. This value, taken together with the value of one non-reducing terminal group per 40—45 xylose residues obtained from the methylation data, suggests that wheat-straw xylan comprises an unbranched chain of xylose residues. Although the presence of some monomethylxylose would be expected from under-methylation of the polysaccharide and/or demethylation during hydrolysis of the methylated polysaccharide, it is interesting that the monomethylxylose consisted almost entirely of the 2-*O*-methyl isomer. Hydrolysis of some aldobiuronic acid units would be expected and it is probable that some of the 2-*O*-methyl-D-xylose was produced in this way.

During the present investigation the results of structural investigations of wheat-straw hemicellulose have been published elsewhere. Without attempting the isolation of a xylan containing no arabinose residues, Adams (*Canad. J. Chem.*, 1952, **30**, 698) studied the structure of a wheat-straw hemicellulose and on the basis of methylation data put forward a structure consisting of a chain of 32 1 : 4-linked D-xylopyranose residues to which are attached 5 L-arabofuranose and 3 D-glucopyruronic acid residues linked through position 3. In addition to the higher proportion of uronic acid residues, it was later suggested by Bishop (*ibid.*, 1953, **31**, 134), investigating the aldobiuronic acid 3-*O*-D-glucopyruronosyl-D-xylose isolated on hydrolysis of the same hemicellulose, that every third uronic acid residue was present as the monomethyl ether. In other respects this hemicelluose appears similar to our wheat-straw xylan : (*a*) the molecular dimensions are of the same order; and (*b*) the side-chains are linked to the main chain through position 3 of the xylose residues. In the light of our present work and of the previous structural investigations on esparto hemicelluloses it seems probable that Adams and Bishop's wheat-straw hemicellulose consists of a mixture of molecular species, ranging from a xylan carrying glucuronic acid but no arabinose side-chains to a highly branched araboxylan.

The present investigation indicates that this xylan, which is only one component of wheat-straw hemicellulose, contains a straight chain of 1 : 4-linked β-D-xylopyranose residues, to one of the non-terminal residues of which a D-glucopyruronic acid residue is linked through position 3. This xylan, therefore, differs in its fine structure from both esparto and pear cell-wall xylans; it resembles pear cell-wall xylan in containing a D-glucuronic acid residue linked to the main chain through position 3, but differs in molecular size and in having an unbranched chain of xylose residues.

Experimental

The following solvents (v/v) were used to separate the sugars and their derivatives: (A) butanol–benzene–pyridine–water (5 : 1 : 3 : 3, top layer), (B) butanol–ethanol–water (4 : 1 : 5, top layer), and (C) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4).

Isolation of Wheat-straw Xylan.—Wheat straw (variety " White Victor," cut in September 1950; 500 g.) was extracted successively with benzene and methanol, and was then delignified by Wise's method (*Ind. Eng. Chem. Anal.*, 1945, **17**, 63). The holocellulose (318 g.) was

extracted with sodium hydroxide solution (4%), the extract acidified with glacial acetic acid, and the crude xylan precipitated by addition of an equal volume of ethanol. The polysaccharide was purified by five successive precipitations of the copper complex formed on addition of Fehling's solution to a solution in aqueous sodium hydroxide (4%). Further purification was effected by two extractions with boiling aqueous ethanol (70%; v/v). The purified xylan (29·6 g.) had $[\alpha]_D^{20}$ −93° (*c*, 0·21 in N-sodium hydroxide) [ash (as sulphate), 0·74; lignin 0·4; uronic anhydride, 3·2; OMe, 0·4%]. Chromatographic examination of the hydrolysate (Hirst and Jones, *J.*, 1949, 1659) in solvent A showed the presence of xylose (88%) and arabinose (0·5%).

Methylation of Wheat-straw Xylan.—Xylan (18·4 g.) was methylated ten times with methyl sulphate and sodium hydroxide, and the product was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 60—65°) mixtures. The two main fractions were further methylated twice with methyl iodide and silver oxide, and the products combined and fractionated as before, to give a main fraction, soluble in boiling chloroform–light petroleum (30 : 70) (9·4 g.) {OMe, 38·2%; $[\alpha]_D^{14}$ −82·7° (*c*, 0·45 in $CHCl_3$)}.

Hydrolysis of Methylated Xylan.—The methylated xylan (5·0 g.) was refluxed successively with methanolic hydrogen chloride (300 c.c.; 0·5%) for 24 hr. and with hydrochloric acid (300 c.c.; 0·5N) for 16 hr. The hydrolysate was neutralised with Amberlite resin IR-4B, and the solution concentrated to a syrup (4·9 g.). Quantitative paper chromatography (Hirst, Hough, and Jones, *J.*, 1949, 298) in solvent B showed the presence of tri-, di-, and mono-methyl xylose in the ratio 1 : 34 : 1.

Separation of Methylated Sugars.—The syrup (4·9 g.) was fractionated on cellulose (90 × 4 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with light petroleum (b. p. 100—120°)–*n*-butanol (7 : 3) saturated with water as eluant, to give four fractions.

Fraction 1. The syrup (144 mg.) did not crystallise and hypoiodite oxidation indicated 79% of aldopentose. A sample (5 mg.) was rehydrolysed and chromatographic examination of the hydrolysate in solvent B showed the presence of 2 : 3-di- and 2 : 3 : 4-tri-*O*-methylxylose. The syrup therefore contained some (*ca.* 20%) methyl 2 : 3-di-*O*-methylxyloside.

The syrup (133 mg.) was rehydrolysed with N-hydrochloric acid (20 c.c.) on the water-bath for 6 hr. {$[\alpha]_D^{20}$ + 23 ⟶ +20° (5 hr., const.)}. After neutralisation with silver carbonate the resulting syrup (130 mg.) was fractionated on cellulose (50 × 1·4 cm.) as before, to give fractions 1*a* (101 mg.) and 1*b* (25 mg.). Fraction 1*a* after recrystallisation from dry ether had m. p. and mixed m. p. (with authentic 2 : 3 : 4-tri-*O*-methyl-D-xylose) 89° and $[\alpha]_D^{16}$ +20° (*c*, 0·9 in H_2O) (Found: OMe, 48·2. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 101°. Fraction 1*b* was identified as 2 : 3-di-*O*-methyl-D-xylose by conversion into its aniline derivative, m. p. and mixed m. p. 122°.

Fraction 2. The syrup (4·18 g.) had $[\alpha]_D^{20}$ +22·9° (*c*, 1·49 in H_2O), n_D^{20} 1·4694 (Found: OMe, 33·9. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). Chromatographic examination showed only 2 : 3-di-*O*-methyl-D-xylose, and hypoiodite oxidation indicated 98·4% purity. The syrup partially crystallised when seeded and had m. p. 77—78°. The sugar was identified by conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 122—123°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 132°.

Fraction 3. The syrup (139 mg.) crystallised and after recrystallisation from methanol had m. p. and mixed m. p. with authentic 2-*O*-methyl-D-xylose 135—136°, and $[\alpha]_D^{19}$ +30° (*c*, 1·6 in H_2O) (Found: OMe, 18·4. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). Chromatographic examination showed only 2-*O*-methyl-D-xylose, and hypoiodite oxidation indicated 99% purity. The identity of the sugar was confirmed by conversion into 2-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 123—124°.

Fraction 4. Elution of the cellulose column with water gave a solid (308 mg.) incompletely soluble in ethanol, methanol, and water. Chromatographic examination in solvent B showed two spots [R_G 0·05 and 0·09—0·10 (dipolar)], and demethylation (Hough, Jones, and Wadman, *J.*, 1950, 1702) gave xylose, 2-*O*-methylxylose, and a trace of 2 : 3-di-*O*-methylxylose. A sample (10 mg.) was heated in a sealed tube at 100° for 6 hr. with methanolic hydrogen chloride, and the solution neutralised with silver carbonate and taken to dryness. An ethereal solution of the resulting syrup was treated with lithium aluminium hydride as described by Chanda, Hirst, and Percival (*loc. cit.*) and chromatographic examination of the hydrolysate showed the presence of 2-*O*-methyl-D-xylose and 2 : 3 : 4-tri-*O*-methyl-D-glucose.

The remainder of fraction 4 (270 mg.), which was contaminated with inorganic material, was purified by dissolution in hot methanol and the resulting syrup was heated on the water-

bath for 5 hr. with N-hydrochloric acid (20 c.c.). After neutralisation with silver carbonate, the hydrolysate was fractionated on filter sheets with solvent B, to give fractions 4*a* (20 mg.) and 4*b* (72 mg.). Fraction 4*a* was shown chromatographically to consist of 2 : 3-di-*O*-methyl-D-xylose only. Fraction 4*b* had $[\alpha]_D^{16}$ +97·5° (*c*, 0·72 in H_2O) (Found : OMe, 32·2%; equiv. 350. Calc. for $C_{15}H_{26}O_{11}$: OMe, 32·4%; equiv., 382).

Fraction 4*b* (13 mg.) was heated with methanolic hydrogen chloride (2 c.c.; 1%) for 6 hr. and neutralised in the usual way. The resulting ester glycoside was reduced with lithium aluminium hydride (Chanda, Hirst, and Percival, *loc. cit.*). The 2-*O*-methyl-D-xylose and 2 : 3 : 4-tri-*O*-methyl-D-glucose formed on hydrolysis of the product were separated chromatographically in solvent B, and estimation by hypoiodite oxidation (Chanda, Hirst, Jones, and Percival, *loc. cit.*) showed them to be present in the ratio of 1·1 : 1. The aldobiuronic acid (53·6 mg.) was converted into the sodium salt and oxidised with 0·25M-sodium metaperiodate solution (3 c.c.), and the periodate consumed after 26 hr. estimated by Fleury and Lange's method (*J. Pharm. Chim.*, 1933, **17**, 107, 196) (Found : 0·8 mol. per $C_{15}H_{26}O_{11}$ unit). Examination of the products of periodate oxidation by the method of Buchanan, Dekker, and Long (*J.*, 1950, 3162) showed that no formic acid was produced.

Chromatographic Examination of the Acidic Fraction from Xylan Hydrolysis.—Xylan (20 g.) was heated with N-sulphuric acid (400 c.c.) at 100° for 7 hr. and the hydrolysate neutralised by passage through a column of Amberlite resin 1R-4B. The resin was washed with 2N-sulphuric acid, and then with water until the eluate was free from sulphate ions. The eluate was neutralised with barium carbonate and the filtrate concentrated to a syrup (A), chromatographic examination of which showed the presence of xylose and an aldopolyuronic acid. Syrup (A) was poured into ethanol, the resulting precipitate was removed, and the solution taken to dryness to give a pale yellow solid (B). The solid (B) which contained xylose and the water-soluble barium salt of an aldopolyuronic acid was hydrolysed with 2N-sulphuric acid (2 c.c.) at 100° for 8 hr. The hydrolysate was partially neutralised with barium carbonate, and the filtrate examined chromatographically, showing the presence of xylose, glucuronic acid, and glucurone but the absence of 4-*O*-methylglucuronic acid.

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Hemicellulose A of Beechwood (Fagus sylvatica).

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Beech hemicellulose A has been shown to contain residues of D-xylose and 4-*O*-methyl-D-glucuronic acid (by conversion into 4-*O*-methyl-D-glucose). The methylated polysaccharide gave on hydrolysis 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3-di-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose, and 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucopyruronosyl)-D-xylose in the molar ratio of 1 : 60 : 7 : 7. A structure is proposed for the hemicellulose which has a straight chain of *ca.* 70 1 : 4-linked β-D-xylopyranose residues with every tenth residue carrying a terminal 4-*O*-methyl-D-glucopyruronic acid residue linked through position 2.

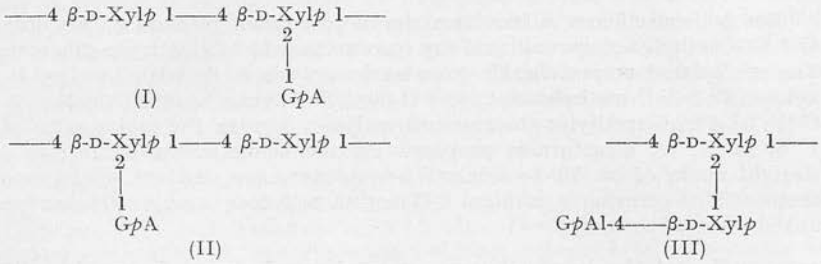
Analytical studies of the hemicelluloses of hard woods have shown that these polysaccharides consist mainly of xylose residues, but, in addition, evidence for the presence of a monomethylhexuronic acid has been obtained (cf. O'Dwyer, *Biochem. J.*, 1939, **33**, 713; 1940, **34**, 149). It was of interest, therefore, to investigate the fine structure of a polysaccharide of this type, particularly in the light of structural studies on the glucuronic acid-containing xylans from the cell-wall of ripe pears (Chanda, Hirst, and Percival, *J.*, 1951, 1240) and wheat straw (Aspinall and Mahomed, preceding paper).

For the present investigation a quantity of the hemicellulose A isolated from European beech wood (*Fagus sylvatica*) was kindly placed at our disposal for structural investigations by Mr. I. R. C. McDonald of the Forest Products Research Laboratory, Princes Risborough (for details of the isolation see McDonald, *J.*, 1952, 3183). It may be recalled that the hemicellulose was obtained by direct alkaline extraction of the extractive-free wood instead of from the " holocellulose " fraction obtained after delignification of the wood. In view of the work of Timell and Jahn (*Svensk Papperstidning*, 1951, **24**, 831), who showed that the polysaccharides of paper birch were degraded during chlorite delignification, and of Jayme and Hank (*Cellulose-chem.*, 1943, **21**, 127) and Bublitz (*Tech. Assoc. Pulp Paper Ind.*, 1951, **34**, 427), who showed that polysaccharides are present in the chlorite delignification liquors of spruce wood, it was felt advisable to carry out these structural investigations on material prepared without delignification.

The beech hemicellulose A, $[\alpha]_D^{20}$ −89·4° (*c*, 0·35 in N-sodium hydroxide), gave on hydrolysis xylose (81·6%) and a trace of rhamnose (*ca.* 0·4%). In addition, the presence of uronic anhydride (9·5%) and of a significant methoxyl content (1·7%) suggested that *O*-methylhexuronic acid residues might also be present. Methylation of the polysaccharide and fractionation of the product gave methylated beech hemicellulose A {$[\alpha]_D^{19}$ −75° (*c*, 0·23 in $CHCl_3$)}. Separation of the products of hydrolysis of the methylated polysaccharide on cellulose gave tri-, di-, and mono-*O*-methylxylose and a tetra-*O*-methylaldobiuronic acid in the molar ratio of 1 : 60 : 7 : 7. The neutral sugars were identified by the formation of crystalline derivatives as 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 3-*O*-methyl-D-xylose respectively.

The acidic fraction was identified as 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-α-D-glucopyruronosyl)-D-xylose in the following way. Reduction of the methyl ester glycoside with lithium aluminium hydride (Lythgoe and Trippett, *J.*, 1950, 1983) followed by hydrolysis and chromatographic separation gave 3-*O*-methyl-D-xylose and 2 : 3 : 4-tri-*O*-methyl-D-glucose in the molar ratio of 1·15 : 1·0. The sugars were identified by conversion into 3-*O*-methyl-D-xylosazone and methyl 2 : 3 : 4-tri-*O*-methyl-β-D-glucopyranoside respectively. That the glucuronic acid residue was linked through position 2 and not position 4 of the xylose residue was shown by the following observations : (*a*) further methylation

of the aldobiuronic acid followed by reduction with lithium aluminium hydride and hydrolysis gave 2 : 3 : 4-tri-*O*-methyl-D-glucose, 3 : 4-di-*O*-methyl-D-xylose (chromatographically separable from 2 : 3-di-*O*-methyl-D-xylose), and 3-*O*-methyl-D-xylose together with a trace of 2 : 3 : 4-tri-*O*-methyl-D-xylose; (*b*) periodate oxidation of the derived 3 : 4-di-*O*-methylxylonic acid gave no formaldehyde, whereas authentic 2 : 3-di-*O*-methyl-D-xylonic acid yielded formaldehyde under similar conditions. The chromatographic identification of a trace of 2 : 3 : 4-tri-*O*-methyl-D-xylose indicates that some aldotriuronic acid was also present in the acidic fraction. The trimethylxylose could only have arisen from a trisaccharide unit derived from the polysaccharide as in (I).



Trisaccharide units (II) and (III) would have given rise to 2 : 3-di-*O*-methylxylose on further methylation followed by hydrolysis. This observation provides further evidence that the uronic acid residues are linked directly to the main chain of xylose residues as in (I) and are not linked through a side-chain as in (III).

The acidic components in beech hemicellulose A were shown to be 4-*O*-methyl-D-glucuronic acid residues by conversion into 4-*O*-methyl-D-glucose. From hydrolysis of the polysaccharide a crude aldobiuronic acid having a significant methoxyl content was isolated. Chromatographic examination showed that vigorous hydrolysis of the aldobiuronic acid yielded xylose, glucuronic acid, and 4-*O*-methylglucuronic acid. Conversion of the aldobiuronic acid into its methyl ester glycoside, followed by reduction with sodium borohydride and hydrolysis of the product gave xylose and 4-*O*-methyl-D-glucose, the latter sugar being identified as the corresponding osazone. 4-*O*-Methyl-D-glucuronic acid has also been isolated from the hydrolysis of aspen wood (*Populus tremuloides*) by Jones and Wise (*J.*, 1952, 2750) and of *Eucalyptus regnans* wood by Stewart and Foster (*Nature*, 1953, **171**, 792). It appears therefore to be a common constituent of wood hemicelluloses.

These results indicate that beech hemicellulose A consists of a linear chain of *ca.* 70 1 : 4-linked D-xylopyranose units with approximately every tenth xylose unit carrying a 4-*O*-methyl-D-glucuronic acid unit linked as a side-chain through position 2. This conclusion is supported by a molecular-weight determination by the isothermal distillation method (by the courtesy of Dr. C. T. Greenwood and Mrs. H. Zinkiewicz) which gave a value of 11,100 $\pm$ 500 (degree of polymerisation, 66—72) for the molecular weight of the methylated hemicellulose. It is difficult to explain the isolation of a large quantity (*ca.* 10%) of monomethyl xylose. Ionophoretic examination of this fraction showed it to consist almost entirely of the 3-*O*-methyl isomer whereas random undermethylation of the polysaccharide and demethylation during hydrolysis would be expected to yield more equal quantities of the two possible isomers. It is interesting that, in the case of wheat-straw xylan (Aspinall and Mahomed, *loc. cit.*) where branching of the glucuronic acid residue occurs through position 3 of the xylose residue, the monomethyl xylose isolated on hydrolysis of the methylated polysaccharide, consisted almost entirely of the 2-*O*-methyl isomer. No methyl ethers of rhamnose were isolated, so it would appear that the rhamnose obtained from hydrolysis of the polysaccharide arose either from a contaminating glycoside or from an associated polysaccharide. It will be recalled that L-rhamnose was isolated by Jones and Wise (*loc. cit.*) from the hydrolysis of aspen wood.

Beech hemicellulose A resembles most closely the polyuronide hemicellulose of New Zealand flax (*Phormium tenax*) (McIlroy, Holmes, and Mauger, *J.*, 1945, 796; McIlroy, *J.*, 1949, 121) in that an acid residue is linked to every tenth residue of the xylan chain.

It is not known, however, whether the acidic residue in the latter case are of D-glucuronic acid or of 4-*O*-methyl-D-glucuronic acid. Beech hemicellulose differs from hemicelluloses of the xylan type previously examined in these laboratories in that branching to the main chain of xylose units occurs through position 2. In the araboxylan from esparto grass (Aspinall, Hirst, Moody, and Percival, *J.*, 1953, 1631) the L-arabofuranose units and in pear cell-wall xylan (Chanda, Hirst, and Percival, *loc. cit.*) and wheat-straw xylan (Aspinall and Mahomed, *loc. cit.*) the D-glucopyruronic acid units are linked to the xylan chain through position 3. The linkage through position 2 has also been found in the hemicelluloses of aspen wood by Jones and Wise (*J.*, 1952, 3389), who isolated 2-*O*-(4-*O*-methyl-D-glucopyruronosyl)-α-D-xylose from the products of hydrolysis. It is clear that hemicelluloses of the xylan type differ markedly amongst themselves in their fine structure and that further investigations are required to unravel their complex relations.

Experimental

The solvents (A, B, and C) used to separate the sugars and their derivatives were those detailed in the preceding paper.

The polysaccharide was prepared, and kindly made available, by Mr. I. R. C. MacDonald of the Forest Products Research Laboratory, Princes Risborough (see *J.*, 1952, 3183). It was received as a fine white powder, $[\alpha]_D^{20}$ −89·4° (*c*, 0·35 in N-NaOH) (Found: OMe, 1·7; uronic anhydride, 9·5; lignin, 1·9%). Chromatographic examination of the hydrolysate (Hirst and Jones, *J.*, 1949, 1659) in solvent A showed the presence of xylose (81·6%) and (*ca.* 0·4%) of rhamnose.

Methylation of Beech Hemicellulose A.—Hemicellulose A (20 g.) was methylated twelve times with methyl sulphate and sodium hydroxide and four times with methyl iodide and silver oxide. The product (14·6 g.; OMe, 38·2%) was fractionated in boiling chloroform–light petroleum (b. p. 60—65°) mixtures to give a main fraction (12·7 g.) {OMe, 38·6%; $[\alpha]_D^{19}$ −75° (*c*, 0·23 in $CHCl_3$), $[\alpha]_D^{18}$ −113° (*c*, 1·25 in *m*-cresol)}.

Hydrolysis of Methylated Hemicellulose A and Separation of Methylated Sugars.—Methylated hemicellulose A (5·0 g.) was hydrolysed successively with 1·5% methanolic hydrogen chloride (500 c.c.) for 14 hr. and N-hydrochloric acid (200 c.c.) at 100° for 12 hr. (constant rotation). Evaporation after neutralisation with silver carbonate yielded a syrup (4·75 g.). The syrup (3·9 g.) was fractionated on cellulose (90 × 4 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with light petroleum (b. p. 100—120°)–*n*-butanol (65 : 35), saturated with water as eluant. Three discrete fractions, 1 (22 mg.), 2 (46 mg.), and 3 (56 mg.), were obtained, but thereafter all fractions were contaminated by the acidic component and the column was eluted with water to give three further fractions, 4 (0·121 g.), 5 (2·988 g.), and 6 (0·321 g.) (recovery, 91%). Partial separation of fraction 4 was effected by dissolving it in water (10 ml.), neutralising the solution with barium carbonate, and exhaustively extracting it with chloroform. The aqueous solution was deionised with Amberlite resin IR-100 to give an acidic fraction 4*a* (63 mg.). The chloroform extract gave fraction 4*b* (35 mg.), which consisted mainly of dimethylxylose but also contained some of the acidic component. Fractions 4*b* and 5 were combined and refractionated on cellulose, with solvent B as eluant, to give fractions 7 (2·108 g.) and 8 (0·390 g.); elution with water gave fraction 9 (0·242 g.) (recovery, 91%).

Examination of the Neutral Fractions.—Fraction 1. The syrup crystallised and had m. p. 63°, $[\alpha]_D^{16}$ −47·3° (*c*, 0·15 in H_2O). The substance was non-reducing and after hydrolysis of a sample with N-hydrochloric acid at 100° for 3 hr., chromatographic examination of the hydrolysate showed only 2 : 3-di-*O*-methylxylose. It is concluded that the substance was methyl 2 : 3-di-*O*-methyl-β-D-xylopyranoside.

Fraction 2. The syrup crystallised completely when seeded with 2 : 3 : 4-tri-*O*-methyl-D-xylose and after recrystallisation from ether had m. p. and mixed m. p. 89°, $[\alpha]_D^{20}$ +22° (*c*, 0·9 in H_2O) (Found: OMe, 48·2. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). Hypoiodite oxidation indicated 99% purity and the derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 98—99°.

Fractions 7 *and* 8*a*. Fraction 7 and fraction 8*a* (see below) were shown to be chromatographically identical and were combined. The syrup had $[\alpha]_D^{15}$ +22·7° (*c*, 1·1 in H_2O), and hypoiodite oxidation indicated 97—98% purity (Found: OMe, 34·5. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The syrup crystallised slowly when seeded with 2 : 3-di-*O*-methyl-D-xylose and, separated on a porous tile, then had m. p. and mixed m. p. 78°. The derived 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 123°.

Fraction 8. Chromatographic examination of the syrup showed the presence of two sugars and separation on cellulose (50 × 3 cm.) with light petroleum (b. p. 100—120°)–*n*-butanol (7 : 3), saturated with water as eluant, gave fractions 8*a* (112 mg.) and 8*b* (252 mg.). Fraction 8*a* was combined with fraction 7 (see above). Fraction 8*b*, a syrup which did not crystallise when seeded with 3-*O*-methyl-D-xylose, had $[\alpha]_D^{20}$ +17° (*c*, 0·8 in H_2O), and hypoiodite oxidation indicated a purity of 97% (Found : OMe, 18·5. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). Paper ionophoresis (Consden and Stanier, *Nature*, 1952, **170**, 1069) showed the presence of 3-*O*-methyl-D-xylose and of a trace of 2-*O*-methyl-D-xylose. The derived 3-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. 136°.

Examination of the Acidic Fractions.—Fractions 4*a*, 6, and 9 were chromatographically similar and were combined. Fraction 3 behaved differently on the chromatogram and was non-acidic (Found : OMe, 34·9. Calc. for $C_{15}H_{26}O_{11}$: OMe, 32·5%), but after hydrolysis with 0·5N-hydrochloric acid (50 c.c.) at 100° for 3 hr. was chromatographically identical with the other acidic fractions, and all the acidic fractions were combined.

The combined acidic fractions (0·682 g.) had $[\alpha]_D^{19}$ +51·5° (*c*, 0·78 in H_2O) (Found : OMe, 32·3% ; equiv., 399. $C_{15}H_{26}O_{11}$ requires OMe, 32·5% ; equiv., 382).

Reduction with Lithium Aluminium Hydride.—The acid (210 mg.) was refluxed with methanolic hydrogen chloride for 6 hr., neutralised, and taken to dryness. The resulting syrup was dissolved in dry ether (75 c.c.), and lithium aluminium hydride (200 mg.) was added during 3 hr. to the refluxing solution. Excess of hydride was destroyed by addition of water, and the solution was acidified with 2N-sulphuric acid and extracted with chloroform (3 × 50 c.c.). The chloroform extract was taken to dryness and the syrup was hydrolysed with 0·5N-hydrochloric acid (50 c.c.) for 7 hr. at 100°. After neutralisation with silver carbonate, the hydrolysate was shown chromatographically to contain sugars travelling at the same rate as 3-*O*-methyl-D-xylose and 2 : 3 : 4-tri-*O*-methyl-D-glucose. The aqueous extract from the reduction was taken to small volume, hydrolysed with N-sulphuric acid (10 c.c.) for 5 hr. at 100°, neutralised with barium carbonate, and taken to a syrup. The syrup was dissolved in methanol, filtered from inorganic material, taken to dryness, and deionised in aqueous solution with Amberlite resins IR-120 and IR-4B. Chromatographic examination of the resulting syrup showed the same two sugars as from the chloroform extract; the sugars from both chloroform and aqueous extracts were therefore combined to give a syrup (163 mg.).

This was fractionated on filter sheets with solvent B, to give fractions *a* (67 mg.) and *b* (79 mg.). Chromatographic and ionophoretic examination of fraction *a* showed only 3-*O*-methylxylose, and the syrup crystallised partly when seeded with 3-*O*-methyl-D-xylose. The crystals had m. p. and mixed m. p. 86—88°, $[\alpha]_D^{20}$ +19·5° (*c*, 0·51 in H_2O) (Found : OMe, 18·5. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). The derived 3-*O*-methyl-D-xylosazone had m. p. and mixed m. p. 172°. Fraction *b* was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the methyl β-D-pyranoside, m. p. and mixed m. p. 92—93°.

Methylation and Reduction with Lithium Aluminium Hydride.—The acid (111 mg.) was converted into the methyl ester glycoside which was methylated twice with methyl iodide and silver oxide. The product was reduced with lithium aluminium hydride as described previously and the resulting syrup was hydrolysed with 0·5N-hydrochloric acid (25 c.c.) for 7 hr. at 100°. After neutralisation with silver carbonate the hydrolysate was taken to dryness, to give a syrup (56 mg.), chromatographic examination of which showed the presence of 2 : 3 : 4-tri-*O*-methylglucose, 3 : 4-di-*O*-methylxylose, 3-*O*-methylxylose, and a trace of 2 : 3 : 4-tri-*O*-methylxylose. Chromatographic separation of the sugars with solvent B, followed by hypoiodite oxidation (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289), showed 2 : 3 : 4-tri-*O*-methylglucose, 3 : 4-di-*O*-methylxylose, and 3-*O*-methylxylose to be present in the molar ratio 1 : 0·95 : 0·48. The solutions resulting from the hypoiodite oxidation of 2 : 3 : 4-tri-*O*-methylglucose, 3 : 4-di-*O*-methylxylose, and an authentic sample of 2 : 3-di-*O*-methyl-D-xylose were each oxidised with sodium metaperiodate solution for 48 hr., the excess of periodate was destroyed with sodium arsenite solution, and the resulting solutions were tested for formaldehyde with phenylhydrazine hydrochloride and potassium ferricyanide (cf. Chanda, Hirst, Percival, and Ross, *J.*, 1952, 1833). Formaldehyde was produced from 2 : 3 : 4-tri-*O*-methylglucose and 2 : 3-di-*O*-methyl-D-xylose but not from 3 : 4-di-*O*-methylxylose.

The Acidic Fraction from the Hydrolysis of Beech Hemicellulose A.—Hemicellulose A (10 g.) was heated with N-sulphuric acid (100 c.c.) at 100° for 6 hr. and, after cooling, the supernatant liquid was neutralised with barium carbonate, filtered, and set aside (I). The residue was heated with N-sulphuric acid (100 c.c.) for a further 4 hr. and the resulting solution was neutralised with barium carbonate, filtered, and combined with (I). The combined solutions were

concentrated to a syrup which was poured into methanol to give a supernatant liquid (II) and a brown solid (III). The solid (III) was reprecipitated from aqueous solution with excess of methanol, washed with methanol, and dried; the washings were added to (II). Chromatographic examination of the reprecipitated solid (III) after removal of barium ions with Amberlite resin IR-120 showed an aldobiuronic acid and xylose to be present.

Further precipitates were obtained from the liquid (II) by the addition of ethanol and concentration of the resulting supernatant liquor to a syrup which was poured into ethanol. These solids were chromatographically similar to solid (III). The combined solids were reprecipitated several times from aqueous solution with methanol and dried (yield, 1·1 g.). A small sample was dried from aqueous solution for analysis (Found: OMe, 4·9. Calc. for barium monomethylaldobiuronate: OMe, 7·6%. Equiv. of resulting acid, 384. Calc. for $C_{12}H_{20}O_{11}$: equiv., 340). The crude barium aldobiuronate (70 mg.) was heated with methanolic 16% hydrogen chloride (5 c.c.) for 20 hr. at 70—80°, neutralised with silver carbonate, and taken to a syrup, which was hydrolysed with 0·5N-hydrochloric acid (2 c.c.) and isolated in the usual manner. Chromatographic examination of the hydrolysate in solvents B and C showed xylose, 4-*O*-methylglucuronic acid, and glucuronic acid to be present.

Reduction with Sodium Borohydride.—Crude barium aldobiuronate (2·3 g.) was converted into the methyl ester glycoside by refluxing methanolic 2% hydrogen chloride (60 c.c.) during 5 hr. After neutralisation with silver carbonate the resulting syrup was dissolved in water (150 c.c.), and sodium borohydride was (1·5 g.) added. The mixture was shaken for 16 hr. at room temperature, acidified with glacial acetic acid to pH 4, and was filtered. The filtrate was made 1·5N with respect to hydrochloric acid and heated for 6 hr. at 100°. After neutralisation with silver carbonate the hydrolysate was deionised with Amberlite resins IR-120 and IR-4B and taken to a syrup. Chromatographic examination showed the presence of xylose, 4-*O*-methylglucose, and a trace of glucose. Part of the syrup (200 mg.) was separated on filter sheets with solvent B, and the 4-*O*-methyl-D-glucose was identified by conversion into 4-*O*-methyl-D-glucosazone, m. p. and mixed m. p. 153—154°. Examination by the *X*-ray powder photograph method, by the kindness of Dr. C. A. Beevers, confirmed the identity of the osazone.

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Cereal Gums. Part I. The Methylation of Barley Glucosans.

By G. O. Aspinall and R. G. J. Telfer.

[Reprint Order No. 5440.]

Two samples of water-soluble lævorotatory glucosan from barley grain have been shown to be composed solely of D-glucose residues. Hydrolysis of the methylated polysaccharides gave 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methyl-D-glucoses. It is concluded that these barley glucosans contain unbranched chains of β-D-glucopyranose residues with approximately equal proportions of 1 : 3- and 1 : 4-linkages.

The cereal gums are the non-starchy water-soluble polysaccharides found in cereal grains (for a review see Preece, *Proc. Eur. Brew. Conv., Brighton*, 1951, 213). Hydrolysis of various cereal gums has shown that residues of D-glucose, D-xylose, and L-arabinose are always present and sometimes in addition smaller amounts of D-galactose and D-mannose. Structural investigations so far carried out have been concerned mainly with fractions rich in pentosan isolated from wheat. Ford and Peat (*J.*, 1941, 856) isolated from wheat grain a water-soluble polysaccharide associated with β-amylase and showed that a highly branched molecule containing L-arabofuranose, D-xylopyranose and D-galactopyranose residues was present. More recently, Perlin (*Cereal Chem.*, 1951, **28**, 370, 382) has shown wheat flours to contain a mixture of water-soluble pentosans and hexosans, the pentosan being an araboxylan containing L-arabofuranose residues attached as side-chains to a main chain of 1 : 4-linked β-D-xylopyranose residues.

A considerable advance in understanding the nature and properties of these water-soluble gums has been made recently by Preece and Mackenzie (*J. Inst. Brewing*, 1952, **58**, 353) who have succeeded in preparing a lævorotatory glucosan free from pentosan by fractionation of barley extracts. Barley grain, previously extracted with boiling 80% aqueous ethanol to remove free sugars and oligosaccharides and to inactivate enzymes, was extracted with water at 40° and the aqueous extracts were fractionated by the addition of ammonium sulphate. A sample of barley glucosan prepared under these mild conditions was kindly placed at our disposal for structural investigations by Professor I. A. Preece and Dr. K. G. Mackenzie of the Heriot-Watt College, Edinburgh. We were also provided with a sample of glucosan isolated from barley extracts modified during the preparation by digestion with an enzymically active barley extract. These glucosans are of great importance in malting as Preece and Mackenzie (*loc. cit.*) have shown that barley enzymes, during germination, produce a rapid hydrolysis so that little of these polysaccharides survives in malt.

Both samples of glucosan (from unmodified and from modified barley) were lævorotatory ($[\alpha]_D^{15}$ −12·5° and −13° respectively in H_2O) and gave on hydrolysis only glucose (96—97%). Hydrolysis of the corresponding methylated glucosans gave mixtures of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methyl-D-glucose together with small quantities of an unidentified di-*O*-methyl-glucose (probably arising from undermethylation of the polysaccharides and/or demethylation during hydrolysis). Both tri-*O*-methyl-D-glucoses were obtained crystalline and the 2 : 4 : 6-isomer was also converted into its aniline derivative. No evidence was obtained for the presence of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose in the hydrolysate of the methylated unmodified glucosan, but chromatographic evidence suggested that a small quantity (<0·5%) of this sugar was present in the hydrolysate of the methylated modified glucosan. The proportions of the 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methyl-D-glucoses were estimated by

following the changes in optical rotation in cold methanolic hydrogen chloride of the hydrolysates from both methylated glucosans (cf. Granichstädten and Percival, *J.*, 1943, 54) : the contributions of minor components being neglected, the two isomers were found to be present in practically equal amounts.

Molecular-weight determinations by the isothermal-distillation method and by osmotic-pressure measurements (by the courtesy of Mr. W. N. Broatch and Dr. C. T. Greenwood) gave values of *ca.* 20,000 (degree of polymerisation *ca.* 100) for the methylated glucosan from unmodified barley. It is difficult, therefore, to understand the complete absence of tetra-*O*-methyl-D-glucose arising from a non-reducing end-group unless a loop structure is postulated for the polysaccharide. However, in the light of our knowledge of the structure of other polysaccharides, such a loop appears unlikely, although it cannot be excluded. Although some evidence was obtained for the presence of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose in the hydrolysate of the methylated glucosan from modified barley, the quantity indicated was considerably smaller than would be expected from a linear molecule of similar size. In all other respects, the two samples of glucosan were closely similar. An ultracentrifugal examination of the glucosan from unmodified barley (by the courtesy of Dr. C. T. Greenwood) indicated the presence of only one component and thus suggested the presence of a single polysaccharide containing 1 : 3- and 1 : 4-linked β-D-glucopyranose residues rather than a mixture of two molecular species.

During the present investigation the preliminary results of structural investigations of the water-soluble polysaccharides of barley grain have been published elsewhere. Gilles, Meredith, and Smith (*Cereal Chem.*, 1952, **29**, 314) showed that the aqueous extract of barley flour (barley gum) gave glucose, xylose, and arabinose on hydrolysis, but did not attempt the isolation of individual components. Fractionation of methylated barley gum gave three components : (*a*) a methylated araboxylan (cf. Perlin, *loc. cit.*) ; (*b*) a methylated poly-α-glucosan, similar to methylated starch ; and (*c*) a methylated poly-β-glucosan ($[\alpha]_D$ −9° in acetone). These workers, however, isolated only 2 : 3 : 6-tri-*O*-methyl-D-glucose from the hydrolysis of the methylated poly-β-glucosan and concluded that this component was probably structurally related to cellulose. It appears more likely that this component was similar to our methylated polysaccharides.

The present investigation indicates that barley glucosans contain unbranched chains of β-D-glucopyranose residues containing approximately equal numbers of 1 : 3- and 1 : 4-linkages. These polysaccharides are therefore structurally similar to lichenin (Meyer and Gürtler, *Helv. Chim. Acta*, 1947, **30**, 751 ; Chanda and Hirst, unpublished work). It is interesting that the enzyme lichenase is found in the seeds of most plants (Karrer and his co-workers, *Helv. Chim. Acta*, 1924, **7**, 144, 159, 916) and that the isolation of lichenin from oat seeds has been claimed (Morris, *J. Biol. Chem.*, 1942, **142**, 881). Preece and Mackenzie's study (*J. Inst. Brewing*, 1952, **58**, 457) of the distribution of pentosans and hexosans in the water-soluble gums of the common cereals (including oats) suggests that the so-called lichenin from oats is similar to the glucosans of barley. Further investigations will be necessary before it can be decided whether these glucosans are truly linear in structure and whether the 1 : 3- and 1 : 4-linkages are regularly or randomly distributed.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems : (*a*) ethyl acetate–acetic acid–water (3 : 1 : 3 ; v/v ; top layer) ; (*b*) butan-1-ol–ethanol–water (4 : 1 : 5 ; v/v ; top layer) ; (*c*) benzene–ethanol–water (169 : 47 : 15 ; v/v ; top layer clarified with ethanol).

Glucosan from unmodified barley

The polysaccharide was prepared, and kindly made available to us, by Professor I. A. Preece and Dr. K. G. Mackenzie (see *J. Inst. Brew.*, 1952, **58**, 353). It had $[\alpha]_D^{15}$ −12·5° (*c*, 1·0 in H_2O) and chromatographic examination of the hydrolysate (Hirst and Jones, *J.*, 1949, 1569) in solvent (*a*) showed the presence of glucose (97%) only.

Methylation of the Glucosan.—The glucosan (6·5 g.) was methylated five times with methyl sulphate and sodium hydroxide solution under nitrogen at room temperature and once with

methyl iodide and silver oxide, and the product (6·8 g.) (Found : OMe, 45·0%) isolated by dissolution in chloroform. Fractionation was effected by refluxing chloroform–light petroleum (b. p. 60—80°) mixtures of different compositions. Two main fractions were obtained and these were combined for subsequent work :

Fraction	% of $CHCl_3$ in solvent	$[\alpha]_D^{17}$ (*c*, 1·0 in $CHCl_3$)	OMe, %	Wt. (g.)
1	20	−5·0°	44·8	2·5
2	25	−5·5	45·0	2·1

Hydrolysis of Methylated Glucosan.—The methylated glucosan (4·1 g.) was refluxed with methanolic 1% hydrogen chloride (200 c.c.) for 6 hr. (constant rotation). Then the solution was neutralised with silver carbonate and concentrated, and the resultant syrup was hydrolysed on the water-bath with 2% hydrochloric acid (160 c.c.) for 3 hr. (constant rotation). After neutralisation with silver carbonate the aqueous solution was concentrated to a syrup (4·27 g.). Chromatographic examination in solvent (*b*) showed the presence of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucoses and a trace of a di-*O*-methylglucose.

Separation of Methylated Sugars and Examination of Fractions.—The syrup (3·79 g.) was fractionated on cellulose (70 × 3 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with light petroleum (b. p. 100—120°)–butanol (7 : 3), saturated with water, as eluant, to give five fractions.

Fraction 1. The syrup (0·808 g.) was non-reducing. A sample was further hydrolysed and chromatographic examination of the hydrolysate showed the presence of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucoses.

Fraction 2. Chromatographic examination of the syrup (0·188 g.) showed the presence of 2 : 3 : 6-tri-*O*-methylglucose together with a small quantity of a substance travelling faster on the chromatogram. Separation on filter sheets with solvent (*c*) gave fractions 2*a* (0·152 g.) and 2*b* (0·030 g.). Fraction 2*a* crystallised and after two recrystallisations from dry ether had m. p. 120—122° (unchanged on admixture with authentic 2 : 3 : 6-tri-*O*-methyl-D-glucose, but depressed on admixture with authentic 2 : 4 : 6-tri-*O*-methyl-D-glucose) and $[\alpha]_D^{16}$ +70° (equil.) (*c*, 1·0 in H_2O) (Found : C, 48·5; H, 7·9; OMe, 41·2. Calc. for $C_9H_{18}O_6$: C, 41·9; H, 8·1; OMe, 41·9%).

Fraction 3. Chromatographic examination of the syrup (2·847 g.) showed 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucose. Fractions 1, 2*b*, and 3 were combined and rehydrolysed to give a syrup (3·51 g.), which had $[\alpha]_D^{16}$ +70° ⟶ +18° (*c*, 1·9 in methanolic 1% hydrogen chloride) and showed only 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucose on the chromatogram.

Fraction 4. The syrup (0·238 g.) crystallised and after two recrystallisations from dry ether had m. p. and mixed m. p. (with authentic 2 : 4 : 6-tri-*O*-methyl-D-glucose) 120—122° and $[\alpha]_D^{17}$ +72° (equil.) (*c*, 1·5 in H_2O) (Found : C, 48·6; H, 7·8; OMe, 41·4. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·1; OMe, 41·9%). The derived 2 : 4 : 6-tri-*O*-methyl-*N*-phenyl-D-glucosylamine had m. p. 144—145° (from ethanol–light petroleum) and 162—164° (from ethyl acetate).

Fraction 5. The syrup (14 mg.), which showed a mixture of tri-, di-, and mono-*O*-methylglucose on the chromatogram, was not examined further.

Estimation of the Relative Proportions of Tri-O-methylglucoses.—A sample (*ca.* 300 mg.) of the hydrolysate of the methylated glucosan was rehydrolysed with 2% hydrochloric acid (30 c.c.) for 7 hr. on the water-bath. After neutralisation of the hydrolysate with silver carbonate, concentration of the solution gave a syrup which showed $[\alpha]_D^{18}$ +61° ⟶ +12° (*c*, 1·32 in methanolic 1% hydrogen chloride). A synthetic mixture of 2 : 3 : 6- (46%) and 2 : 4 : 6-tri-*O*-methyl-D-glucose (54%) showed $[\alpha]_D$ +71° ⟶ +18° (*c*, 1·0 in methanolic 1% hydrogen chloride).

Glucosan from modified barley

The glucosan had $[\alpha]_D^{15}$ −13° (*c*, 0·88 in H_2O) and chromatographic examination of the hydrolysate (Hirst and Jones, *loc. cit.*) in solvent (*a*) showed the presence of glucose (96%) only.

Methylation of the Glucosan.—The glucosan (4·5 g.) was methylated six times with methyl sulphate and sodium hydroxide solution under nitrogen at room temperature, and twice with methyl iodide and silver oxide. The product (3·7 g.) (OMe, 45·0%), $[\alpha]_D^{16}$ −5·3° (*c*, 1·0 in $CHCl_3$), was purified by precipitation from chloroform solution with light petroleum (b. p. 40—60°).

Hydrolysis of Methylated Glucosan.—The methylated glucosan (3·0 g.) was refluxed with methanolic 1% hydrogen chloride (175 c.c.) for 7 hr. (constant rotation). After neutralisation with silver carbonate the residual syrup was hydrolysed on the water-bath with 2% hydrochloric acid (150 c.c.) for 10 hr., the hydrolysate was neutralised with silver carbobate and the solution was taken to dryness to give a syrup (2·96 g.). Paper chromatographic examination in solvents

(*b*) and (*c*) showed the presence of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucose together with traces of tetra-*O*-methylglucose and a di-*O*-methylglucose.

Separation of Methylated Sugars and Examination of Fractions.—The syrup (2·68 g.) was fractionated on cellulose (70 × 3 cm.) with light petroleum (b. p. 100—120°)–butanol (7 : 3), saturated with water, as eluant, to give six fractions.

Fraction 1. Chromatographic examination of the syrup (12 mg.) showed the presence of 2 : 3 : 4 : 6-tetra-*O*-methylglucose. The syrup, however, did not crystallise when seeded with authentic tetra-*O*-methyl-D-glucose and chromatographic examination after further hydrolysis showed that it also contained an approximately equal amount of methyl 2 : 3 : 6-tri-*O*-methyl-D-glucoside.

Fraction 2. The syrup (0·41 g.) was non-reducing and was rehydrolysed with 2% hydrochloric acid (20 c.c.). The syrupy hydrolysate (0·40 g.) was examined chromatographically and shown to contain a mixture of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucose.

Fraction 3. The syrup (1·15 g.) was chromatographically pure and crystallised. Two recrystallisations from dry ether gave 2 : 3 : 6-tri-*O*-methyl-D-glucose, m. p. and mixed m. p. 122—123°, $[\alpha]_D^{17}$ +71° (equil.) (*c*, 1·1 in H_2O) (Found : OMe, 41·5. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction 4. The syrup (0·24 g.) was shown chromatographically to contain a mixture of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucose.

Fraction 5. The syrup (0·78 g.) crystallised and chromatographic examination showed the presence of 2 : 4 : 6-tri-*O*-methylglucose together with some 2 : 3 : 6-tri-*O*-methylglucose. Three recrystallisations from dry ether gave 2 : 4 : 6-tri-*O*-methyl-D-glucose, m. p. and mixed m. p. 120—122°, $[\alpha]_D^{15}$ +73° (equil.) (*c*, 2·0 in H_2O). The identity of the sugars was confirmed by conversion into 2 : 4 : 6-tri-*O*-methyl-*N*-phenyl-D-glucosylamine, m. p. and mixed m. p. 162—164°.

Fraction 6. After separation of fraction 5 the column was eluted with butanol, partly saturated with water, to give a syrup (11 mg.) which travelled on the chromatogram at the rate of a di-*O*-methylglucose.

Estimation of the Relative Proportions of Tri-O-methylglucoses.—A sample (*ca.* 100 mg.) of the hydrolysate of the methylated glucosan was rehydrolysed with 2% hydrochloric acid (15 c.c.) for 4 hr. on the water-bath. After neutralisation of the hydrolysate with silver carbonate, concentration gave a syrup which showed $[\alpha]_D^{16}$ +61° ⟶ +15° (*c*, 0·87 in methanolic 1% hydrogen chloride).

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THE METHYL ETHERS OF HEXURONIC ACIDS

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Contents

I. Introduction

Hexuronic acid residues occur in a large number of different polysaccharides, and it is in relation to the detailed chemical structure of such substances that the methyl ethers of uronic acids are of great importance. This article is restricted to a discussion of the methyl ethers of the naturally occurring D-glucuronic, D-galacturonic, and D-mannuronic acids, and their derivatives. In addition, because of their importance in structural determinations, particularly of plant gums and mucilages,[1] a section has been included dealing with the methyl ethers of aldobiouronic acids.

D-Glucuronic acid (or its 4-methyl ether) occurs in combination with several different sugar residues: with D-galactose and D-mannose in plant

(1) J. K. N. Jones and F. Smith, *Advances in Carbohydrate Chem.*, **4**, 243 (1949).

gums, with D-xylose in wood and straw hemicelluloses, and with D-glucose, L-rhamnose, D-glucosamine, and D-galactosamine in various mucopolysaccharides.[2] D-Galacturonic acid occurs in pectic acid, and, combined with L-rhamnose and D-galactose, in seed mucilages and plant gums. D-Mannuronic acid is only known to occur as the sole constituent of the seaweed mucilage, alginic acid.[2a]

Although the methods employed in the synthesis and derivation of structure of the methyl ethers of uronic acids are closely similar to those described in previous articles of this series for the methyl ethers of aldoses, the differences occasioned by their acidic character will be discussed. For synthetic purposes, the methods of Haworth and of Purdie have been used for the introduction of *O*-methyl groups. The Haworth method, used with caution, is suitable for the direct methylation either of reducing uronic acids or their non-reducing methyl glycosides; in both cases, the product is the sodium salt of the methyl glycoside of the methylated uronic acid, from which the methyl glycoside methyl ester may be formed either by methanolysis or by esterification of the free acid with diazomethane. The Purdie reagents, silver oxide and methyl iodide, are best used only when the reducing group is protected from oxidation. The latter reagents do not open pre-formed lactone rings, a fact made use of in the synthesis of 2,5-di-*O*-methyl-D-glucuronic acid.

Two general routes have been used for the synthesis of methyl ethers of uronic acids: (a) direct methylation of the uronic acid or uronic acid derivative; and (b) the preparation of the methyl glycoside of the corresponding methylated hexose, followed by selective oxidation of the primary alcoholic group at C6 to give the hexuronic acid. For the oxidation, alkaline permanganate has been most commonly used, but the recent use of catalytic oxidation (over platinum) for the synthesis of glycosiduronic acids from the corresponding hexosides[3–5] suggests that this method might be extended, particularly for compounds with labile substituents.

Methylated uronic acids are usually separated from methylated aldoses by virtue of the insolubility of their barium salts in chloroform. Recently, 4-*O*-methyl-D-glucuronic acid has been separated from a mixture of non-acidic reducing sugars by absorption on a weakly basic

(2) M. Stacey, *Advances in Carbohydrate Chem.*, **2**, 161 (1947).

(2a) *See* T. Mori, *Advances in Carbohydrate Chem.*, **8**, 315 (1953).

(3) C. L. Mehltretter, B. H. Alexander, R. L. Mellies and C. E. Rist, *J. Am. Chem. Soc.*, **53**, 2424 (1951); C. L. Mehltretter, *Advances in Carbohydrate Chem.*, **8**, 231 (1953).

(4) C. A. Marsh, *J. Chem. Soc.*, 1578 (1952).

(5) S. A. Barker, E. J. Bourne and M. Stacey, *Chemistry & Industry*, 970 (1951).

anion-exchange resin.[6] However, evidence that reducing sugars are considerably degraded on strongly basic resins[7,8,8a] shows that caution is required in the use of basic ion-exchange resins with sugars carrying an unprotected reducing group. Mixtures of uronic acids have usually been separated by fractional distillation of their methyl glycoside methyl esters. Although methylated uronic acids have been identified by paper chromatography, quantitative separation by partition chromatography on cellulose has not been used extensively.

The methyl ethers of uronic acids have been isolated as sirups, and only two free acids have been obtained in the crystalline state. In most cases, crystalline derivatives have resulted on conversion to one of the methyl glycosides, or to the amide or methyl ester of a methyl glycoside. Oxidation of the uronic acid to the corresponding aldaric acid has often proved a convenient method of identification, as the same derivative may be prepared from the corresponding methylated aldose. Alternatively, identification has been achieved by reduction of the carboxyl group at C6, to give the corresponding hexose. This reduction has been achieved easily by using lithium aluminum hydride to reduce the methyl glycoside methyl ester of the uronic acid to the methyl glycoside of the derived hexose.[9,10] Sodium borohydride can also be utilized, and can be employed in aqueous solution.[10a]

II. The Methyl Ethers of D-Glucuronic Acid

1. *3-O-Methyl-D-glucuronic Acid*

3-*O*-Methyl-D-glucuronic acid was first synthesized by Levene and Meyer[11] by reduction of the *O*-methyl-D-glucarolactone formed on oxidation (HNO_3) of 3-*O*-methyl-D-glucose. A comparison of the properties of the *p*-bromophenylhydrazine salt of the *p*-bromophenylosazone of the *O*-methyl-D-glucuronic acid with the corresponding derivative of D-glucuronic acid indicated that the methyl group was in position 3. Furthermore, the *O*-methyl-D-glucose gave, on ascent of the series, a dextrorotatory *O*-methyl-D-glucoheptonolactone, which, according to Hudson's lactone rule, was the 4-*O*-methyl-δ-heptonolactone and not the 5-*O*-methyl-γ-heptonolactone. Recently, the uronic acid has been synthesized

(6) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 796 (1952).
(7) J. D. Phillips and A. G. Pollard, *Nature*, **171**, 41 (1953).
(8) A. C. Hulme, *Nature*, **171**, 610 (1953).
(8a) L. Rebenfeld and E. Pacsu, *J. Am. Chem. Soc.*, **75**, 4370 (1953).
(9) B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 1983 (1950).
(10) M. Abdel-Akher and F. Smith, *Nature*, **166**, 1037 (1950).
(10a) M. L. Wolfrom and Kimiko Anno, *J. Am. Chem. Soc.*, **74**, 5583 (1952).
(11) P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, **60**, 173 (1924).

by catalytic oxidation of 1,2-*O*-isopropylidene-3-*O*-methyl-D-glucose, followed by removal of the isopropylidene residue.[4]

2. *4-O-Methyl-D-glucuronic Acid*

Despite indications (from analytical data) that a monomethyl ether of a uronic acid occurs in the hydrolyzates of several polysaccharides, definite evidence for the presence of 4-*O*-methyl-D-glucuronic acid did not appear until 1948, when White found it in mesquite-gum hydrolyzate.[12] Confirmation of its presence therein has since[13] appeared, and it has also been found in the hydrolyzates from gum myrrh,[6] aspen wood,[14] and *Eucalyptus regnans* wood.[15]

The structure of the uronic acid was established as follows: (a) periodate oxidation, followed by bromine oxidation, gave 2-hydroxy-3-methoxy-L-*erythro*-succinic acid;[12,13] (b) reduction of the methyl glycoside methyl ester with lithium aluminum hydride, followed by hydrolysis, gave 4-*O*-methyl-D-glucose.[13]

3. *2,3-Di-O-methyl-D-glucuronic Acid*

2,3-Di-*O*-methyl-D-glucuronic acid has been isolated from the hydrolysis products of several methylated plant gums (such as damson gum[16,17] and gum arabic[18]) and of the methylated bacterial polysaccharides of *Rhizobium radicicolum*[19] and *Pneumococcus* Type II.[20] Its structure was established as follows: (a) further methylation gave the known 2,3,4-tri-*O*-methyl-D-glucuronic acid;[18] (b) oxidation (HOBr), followed by treatment with methanolic hydrogen chloride, gave 2,3-di-*O*-methyl-D-glucaro-1,4-lactone methyl ester,[16,18] which was also prepared from 2,3-di-*O*-methyl-D-glucose.

4. *2,5-Di-O-methyl-D-glucuronic Acid*

Pryde and Williams[21] first synthesized a derivative of 2,5-di-*O*-methyl-D-glucuronic acid. Methylation of D-glucuronolactone with

(12) E. V. White, *J. Am. Chem. Soc.*, **70,** 367 (1948).
(13) F. Smith, *J. Chem. Soc.*, 2646 (1951).
(14) J. K. N. Jones and L. E. Wise, *J. Chem. Soc.*, 2750 (1952).
(15) C. M. Stewart and D. H. Foster, *Nature*, **171,** 792 (1953); *Austral. J. Chem.*, **6,** 431 (1953).
(16) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1482 (1939).
(17) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 506 (1946).
(18) F. Smith, *J. Chem. Soc.*, 1035 (1940).
(19) Elsa Schlüchterer and M. Stacey, *J. Chem. Soc.*, 776 (1945).
(20) P. W. Kent, *Chemistry & Industry*, 1176 (1952); K. Butler and M. Stacey, *ibid.*, 37 (1953).
(21) J. Pryde and R. T. Williams, *Biochem. J.*, **27,** 1205 (1933).

methyl iodide and silver oxide gave a crystalline "trimethyl glucurone" which was tentatively supposed to be methyl 2,4-di-*O*-methyl-α-D-glucopyranosidurono-6,3-lactone. Reeves[22] considered the "trimethyl glucurone" to be the corresponding 2,5-isomer, as it underwent rapid mutarotation in cold methanolic hydrogen chloride to give a low-melting form (probably the methyl β-D-glycoside), and the rapid rate of hydrolysis in aqueous hydrochloric acid was that characteristic of methyl furanosides. The presence of a furanose ring was conclusively proved by Smith[23] who methylated the "trimethyl glucurone" with dimethyl sulfate and sodium hydroxide, and, after esterification with diazomethane, isolated methyl (methyl 2,3,5-tri-*O*-methyl-α-D-glucosid)uronate, which was distinct from the 2,3,4-isomer and which was converted to the characteristic 2,3,5-tri-*O*-methyl-D-glucaro-1,4-lactone methyl ester. The presence of methoxyl groups at C2 and C5 was proved by the conversion of the "trimethyl glucurone" to the diamide of 2,5-di-*O*-methyl-D-glucaric acid, which gave a negative Weerman test. The "trimethyl glucurone" was thus shown to be methyl 2,5-di-*O*-methyl-α-D-glucosidurono-6,3-lactone.

The anomeric, methyl β-D-glycoside lactone has also been synthesized by methylation of methyl β-D-glucofuranosidurono-6,3-lactone.[24] "Trimethyl glucurone," formed by methylation of D-glucuronolactone with methyl iodide and silver oxide, is usually accompanied by some 2,5-di-*O*-methyl-Δ^4-D-glucaro-6,3-lactone methyl ester ("trimethyl glucuralone"), an oxidation product.

5. *3,4-Di-O-methyl-D-glucuronic Acid*

Derivatives of this uronic acid were first isolated by Lythgoe and Trippett[9] in their study of the structure of glycyrrhizic acid. Methanolysis of methylated glycyrrhizic acid gave the dimethyl ester of the pentamethyl ether of a methyl "dihexuronoside," in addition to the aglycon. Formic-acid hydrolysis of the glycoside, followed by successive treatment with methanolic hydrogen chloride and methanolic ammonia, gave the amide of a methyl di-*O*-methyl-D-glucosiduronic acid. That this amide was derived from 3,4-di-*O*-methyl-D-glucuronic acid was proved as follows: (a) conversion to the amide of methyl 2,3,4-tri-*O*-methyl-αβ-D-glucosiduronic acid showed the presence of a pyranose ring; (b) the positions of the methyl substituents were shown by reduction of the methyl glycoside methyl ester with lithium aluminum hydride, the resultant methyl glycoside giving 3,4-di-*O*-methyl-D-glucose on hydrolysis. 3,4-Di-

(22) R. E. Reeves, *J. Am. Chem. Soc.*, **62**, 1616 (1940).
(23) F. Smith, *J. Chem. Soc.*, 584 (1944).
(24) L. N. Owen, S. Peat and W. J. G. Jones, *J. Chem. Soc.*, 339 (1941).

O-methyl-D-glucuronic acid has been isolated in the crystalline state from the hydrolyzate of methylated sapote gum.[24a]

6. *2,3,4-Tri-O-methyl-D-glucuronic Acid*

2,3,4-Tri-*O*-methyl-D-glucuronic acid was first isolated by Challinor, Haworth and Hirst[25] from the hydrolyzate of the methylated aldobiouronic acid derived from gum arabic. Treatment of the uronic acid with dimethyl sulfate and sodium hydroxide gave the methyl β-D-pyranoside which was hydrolyzed at the same rate as is methyl β-D-glucopyranoside, indicating the presence of a pyranose ring. Proof of the positions of the methyl ether groups was obtained by oxidation (HNO_3) of derivatives of the tri-*O*-methyl-D-glucuronic acid, methanolysis and distillation of the product giving the methyl ester of 2,3,4-tri-*O*-methyl-D-glucaro-1,5-lactone, which was similarly derived from 2,3,4-tri-*O*-methyl-D-glucose.[26,27]

The uronic acid has been isolated from the hydrolyzates of several methylated plant gums[1] and of the methylated aldobiouronic acids derived therefrom. The uronic acid and its derivatives have been synthesized in the following ways: (a) the methyl β-D-pyranoside was synthesized by direct methylation of D-glucuronolactone with dimethyl sulfate and sodium hydroxide; (b) methylation of methyl (methyl D-glucopyranosid)uronate with methyl iodide and silver oxide gave the methyl glycoside methyl ester (mainly the α-form);[24] (c) the methyl β-D-pyranoside has also been synthesized by oxidation of methyl 2,3,4-tri-*O*-methyl-β-D-glucoside with alkaline permanganate.[28]

7. *2,3,5-Tri-O-methyl-D-glucuronic Acid*

The methyl α-glycoside methyl ester of this sugar was prepared by the methylation of methyl (methyl 2,5-di-*O*-methyl-α-D-glucosid)uronate with dimethyl sulfate and sodium hydroxide, followed by esterification with diazomethane. The constitution follows from the method of synthesis.

III. The Methyl Ethers of D-Galacturonic Acid[29]

1. *2-O-Methyl-D-galacturonic Acid*

Derivatives of 2-*O*-methyl-D-galacturonic acid were first synthesized by Jones and Stacey,[30] who converted methyl (methyl α-D-galactopy-

(24a) E. V. White, *J. Am. Chem. Soc.*, **75**, 4692 (1953).
(25) S. W. Challinor, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 258 (1931).
(26) A. Robertson and R. B. Waters, *J. Chem. Soc.*, 1709 (1931).
(27) W. Charlton, W. N. Haworth and R. W. Herbert, *J. Chem. Soc.*, 2855 (1931).
(28) F. Smith, M. Stacey and P. I. Wilson, *J. Chem. Soc.*, 131 (1944).
(29) In addition to the compounds described below, the crystalline methyl

ranosid)uronate into its 3,4-*O*-isopropylidene derivative, which was methylated with methyl iodide and silver oxide, and the product then partially hydrolyzed to remove the isopropylidene residue. The resulting sirupy methyl (methyl 2-*O*-methyl-α-D-galactopyranosid)uronate was characterized as the derived amide. This synthesis has recently been modified, and the yield improved, by Edington and Percival.[31] 2-*O*-Methyl-D-galacturonic acid has been isolated from the hydrolyzates of methylated cholla gum[32] and methylated *Sterculia setigera* gum.[33] The structure of the uronic acid follows from the method of synthesis.

2. *2,3-Di-O-methyl-D-galacturonic Acid*

2,3-Di-*O*-methyl-D-galacturonic acid has been isolated from the hydrolyzate of methylated pectic acid[34–36] and of methylated slippery-elm mucilage.[37] Both methyl furanosides and both methyl pyranosides were isolated from the methanolysis of methylated pectic acid. Contrary to normal experience, this di-*O*-methyluronic acid shows a marked tendency to form a methyl furanoside with hot methanolic hydrogen chloride,[34] and prolonged methanolysis is necessary for forming the methyl pyranosides.[35] Further methylation of the methyl glycosides gave derivatives of 2,3,5-[34,38] and 2,3,4[35]-tri-*O*-methyl-D-galacturonic acids, respectively, thus indicating the absence of methoxyl groups at C4 and C5. Conclusive evidence for the presence of methoxyl groups at C2 and C3 was obtained by oxidation (HNO_3) of the original, methylated uronic acid, followed by methanolysis and distillation, to give the methyl ester of 2,3-di-*O*-methylgalactaro-1,4-lactone, which was also prepared from 2,3-di-*O*-methyl-D-galactose.[34]

3. *2,4-Di-O-methyl-D-galacturonic Acid*

A di-*O*-methyl-D-galacturonic acid was isolated by Hirst, Hough and Jones[39] from the hydrolysis products of the mixture of methylated aldo-

β-glycoside methyl ester of a mono-*O*-methyl-D-galacturonic acid has been isolated from the methanolysis of methylated tragacanthic acid [Sybil P. James and F. Smith, *J. Chem. Soc.*, 739 (1945)]. The presence of a pyranose ring was shown, as further methylation gave the corresponding derivative of 2,3,4-tri-*O*-methyl-D-galacturonic acid, but the position of the methyl group has not been determined.

(30) J. K. N. Jones and M. Stacey, *J. Chem. Soc.*, 1340 (1947).
(31) R. A. Edington and Elizabeth E. Percival, *J. Chem. Soc.*, 2473 (1953).
(32) F. Brown, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1761 (1949).
(33) L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 1199 (1950).
(34) Sybil P. Luckett and F. Smith, *J. Chem. Soc.*, 1106 (1940).
(35) Sybil P. Luckett and F. Smith, *J. Chem. Soc.*, 1506 (1940).
(36) G. H. Beavan and J. K. N. Jones, *J. Chem. Soc.*, 1218 (1947).
(37) R. E. Gill, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1025 (1946).
(38) Sybil P. Luckett and F. Smith, *J. Chem. Soc.*, 1114 (1940).
(39) E. L. Hirst, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 3145 (1949).

biouronic acids from *Sterculia setigera* gum. Although no crystalline derivatives were prepared, the authors suggested that the substance was either 2,4- or 3,4-di-*O*-methyl-D-galacturonic acid, since treatment with cold methanolic hydrogen chloride caused an upward change in optical rotation, thus indicating the presence of a methoxyl group at C4, as the optical rotation of a solution of 2,3-di-*O*-methyl-D-galacturonic acid in methanolic hydrogen chloride changes from a positive to a negative value. In view of the subsequent isolation of 2-*O*-methyl-D-galacturonic acid from the hydrolyzate of methylated *Sterculia setigera* gum,[33] it seems probable that the original, methylated uronic acid is the 2,4-isomer.

4. *3,4-Di-O-methyl-D-galacturonic Acid*

This uronic acid has been synthesized by Edington and Percival.[31] Methyl (methyl 2-*O*-tosyl-α-D-galactopyranosid)uronate, prepared from methyl (methyl 3,4-*O*-isopropylidene-α-D-galactosid)uronate by tosylation followed by methanolysis to remove the isopropylidene residue, was methylated with methyl iodide and silver oxide. The product, methyl (methyl 3,4-di-*O*-methyl-2-*O*-tosyl-α-D-galactosid)uronate, was treated with aqueous methanolic sodium hydroxide to give the crystalline methyl 3,4-di-*O*-methyl-α-D-galactosiduronic acid. The constitution of the uronic acid follows from this method of synthesis, and is confirmed by the following observations: (a) further methylation of the methyl α-glycoside methyl ester gave the corresponding derivative of 2,3,4-tri-*O*-methyl-D-galacturonic acid; (b) oxidation (HOBr) of the uronic acid, followed by esterification, gave the optically inactive dimethyl ester of 3,4-di-*O*-methylgalactaric acid.

5. *2,3,4-Tri-O-methyl-D-galacturonic Acid*

Levene and Kreider[40] first synthesized the methyl α-glycoside methyl ester of 2,3,4-tri-*O*-methyl-D-galacturonic acid by methylating methyl α-D-galactopyranosiduronic methyl ester with methyl iodide and silver oxide. An alternative method of synthesis, by which both methyl glycoside methyl esters have been prepared, involves oxidation of the methyl α- and β-glycosides of 2,3,4-tri-*O*-methyl-D-galactose.[28,35] 2,3,4-Tri-*O*-methyl-D-galacturonic acid was obtained as a crystalline monohydrate by Tipson[41] on hydrolysis of the methyl α-D-glycoside methyl ester. The constitution of the uronic acid follows from the methods employed in its synthesis and from the following observations: (a) oxidation (HNO_3) of the methyl α-D-glycoside methyl ester yielded L-*arabo*-trimethoxyglutaric

(40) P. A. Levene and L. C. Kreider, *J. Biol. Chem.*, **120**, 597 (1937).
(41) R. S. Tipson, *J. Biol. Chem.*, **125**, 341 (1938).

acid;[40] (b) the methyl α-D-glycoside methyl ester was reduced, by catalytic hydrogenation over copper chromite, and the resulting glycoside was hydrolyzed to give 2,3,4-tri-*O*-methyl-D-galactose.[42] 2,3,4-Tri-*O*-methyl-D-galacturonic acid has been isolated from the hydrolyzates of several methylated aldobiouronic acids (from linseed mucilage,[43] slippery-elm mucilage,[44] and *Sterculia setigera* gum[39]).

6. *2,3,5-Tri-O-methyl-D-galacturonic Acid*

The methyl β-D-glycoside methyl ester of this uronic acid was first prepared by Luckett and Smith,[34] who further methylated the main fraction of the methanolysis product from methylated pectic acid. Oxidation (HNO_3), followed by esterification, gave 2,3,5-tri-*O*-methyl-D-galactaro-1,4-lactone methyl ester, and the derived diamide gave a negative Weerman test. Confirmation of the structure of the uronic acid was obtained by synthesis of the methyl β-D-glycoside methyl ester.[38] Methyl 6-*O*-trityl-D-galactofuranoside was methylated with methyl iodide and silver oxide, the trityl residue was removed, and the methyl tri-*O*-methyl-D-galactoside was oxidized with alkaline permanganate. Methanolysis of the resulting product led to the isolation of the crystalline methyl (methyl 2,3,5-tri-*O*-methyl-β-D-galactosid)uronate.

IV. The Methyl Ethers of D-Mannuronic Acid

1. *2,3-Di-O-methyl-D-mannuronic Acid*

2,3-Di-*O*-methyl-D-mannuronic acid has been isolated from the hydrolyzate of methylated alginic acid.[45] The presence of methoxyl groups at C2 and C3 was shown by the formation of *erythro*-dimethoxysuccinic acid from the derived di-*O*-methylmannaric acid, by periodate oxidation followed by bromine oxidation. Confirmation of the structure of the uronic acid was obtained recently by Chanda, Hirst, Percival and Ross[46] who hydrolyzed methylated alginic acid with anhydrous formic acid, and reduced the derived mixture of methyl ester methyl glycosides with lithium aluminum hydride. After hydrolysis and chromatographic separation, the main product was identified as 2,3-di-*O*-methyl-D-mannose.

(42) P. A. Levene, R. S. Tipson and L. C. Kreider, *J. Biol. Chem.*, **122**, 199 (1937).
(43) R. S. Tipson, C. C. Christman and P. A. Levene, *J. Biol. Chem.*, **128**, 609 (1939).
(44) R. E. Gill, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1469 (1939).
(45) E. L. Hirst, J. K. N. Jones and Winifred O. Jones, *J. Chem. Soc.*, 1880 (1939).
(46) S. K. Chanda, E. L. Hirst, E. G. V. Percival and A. G. Ross, *J. Chem. Soc.*, 1833 (1952).

2. *2,3,4-Tri-O-methyl-D-mannuronic Acid*

Ault, Haworth and Hirst[47] first synthesized the methyl α-D-glycoside methyl ester of 2,3,4-tri-*O*-methyl-D-mannuronic acid by successive treatment of potassium (methyl α-D-mannopyranosid)uronate with dimethyl sulfate and sodium hydroxide, and then methyl iodide and silver oxide. Although no crystalline derivatives were isolated, there is little doubt about its structure, since the authors[48] subsequently proved the presence of a pyranose ring in the starting material for the synthesis, methyl 2,3-*O*-isopropylidene-α-D-mannoside. The uronic acid has also been synthesized by Smith, Stacey and Wilson,[28] who oxidized methyl 2,3,4-tri-*O*-methyl-α-D-mannoside with alkaline permanganate and obtained the sirupy acid on hydrolysis of the product.

Proof of the structure of the uronic acid was obtained by Hirst, Jones and Jones,[45] who isolated the methyl glycoside methyl ester by methylation of the products from the graded methanolysis of alginic acid. The tri-*O*-methyl-D-mannuronic acid was converted to the diamide of 2,3,4-tri-*O*-methyl-D-mannaric acid, which had previously been prepared from 2,3,4-tri-*O*-methyl-D-mannose.[49] Evidence that 2,3,4-tri-*O*-methyl-D-mannuronic acid occurs in the hydrolyzate of methylated alginic acid was obtained by Chanda, Hirst, Percival and Ross,[46] who identified 2,3,4-tri-*O*-methyl-D-mannose after reduction of the methyl ester methyl glycosides with lithium aluminum hydride.

V. The Methyl Ethers of Aldobiouronic Acids

The occurrence in many polyglycosiduronic acids of relatively resistant linkages, usually those between uronic acid residues and adjacent residues, has resulted in the isolation of several aldobiouronic acids on graded hydrolysis of acidic polysaccharides. The isolation of such aldobiouronic acids, and subsequent conversion to their fully methylated derivatives, has become a standard procedure in structural studies on polyglycosiduronic acids, especially those of plant gums and mucilages. In other cases, partially methylated aldobiouronic acids have been isolated from the hydrolyzates of methylated polysaccharides. The sources and methods of isolation of the methyl ethers of aldobiouronic acids so far examined are given in Table I. Some properties of derivatives are recorded in Table V.

Despite the frequent isolation of the same methylated aldobiouronic acid from more than one source, the direct identification of such a com-

(47) R. G. Ault, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 517 (1935).

(48) R. G. Ault, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1012 (1935).

(49) W. N. Haworth, E. L. Hirst, F. Isherwood and J. K. N. Jones, *J. Chem. Soc.*, 1878 (1939).

TABLE I
The Methyl Ethers of Aldobiouronic Acids

Substance	*Source*	*Method of isolation*[a]	*Configuration at biose link*	*References*
2,3,6-Tri-*O*-methyl-D-galactose-(4 → 1) 2,3,4-tri-*O*-methyl-D-galactosiduronic acid	Karaya gum	B	—	50
3,4-Di-*O*-methyl-L-rhamnose-(2 → 1) 2,3,4-tri-*O*-methyl-D-galactosiduronic acid	Linseed mucilage	B	—	43
	Slippery-elm mucilage (bark of *Ulmus fulva*)	B	—	44
	Plantago ovata mucilage (seed)	B	—	51
	Plantago arenaria mucilage (seed)	B	—	52
	Sterculia setigera gum	B	—	39
	Karaya gum	B	—	50 See 70a, 70b
2,4-Di-*O*-methyl-D-galactose-(3 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Mesquite gum	C	—	53
2,4,6-Tri-*O*-methyl-D-galactose-(3 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Mesquite gum	D	—	53
2,3,6-Tri-*O*-methyl-D-galactose-(4 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Mesquite gum	B	—	54
	Grapefruit gum	B	—	55
	Lemon gum	B	—	55
D-Galactose-(6 → 1) 4-*O*-methyl-D-glucosiduronic acid	Mesquite gum	A	β	13, 56
2,4-Di-*O*-methyl-D-galactose-(6 → 1) 2,3-di-*O*-methyl-D-glucosiduronic acid	Egg-plum gum	C	—	57
2,3,4-Tri-*O*-methyl-D-galactose-(6 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Gum arabic	B	β	25, 58, 59
		C	β	60
	Mesquite gum	B	β	54, 56
	Egg-plum gum	B	—	61
	Almond-tree gum	B	—	62
	Peach gum	B	—	63
	Black-wattle gum	B	—	64 See 70c
2,3,6-Tri-*O*-methyl-D-glucose-(4 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Pneumococcus Type III specific polysaccharide	B	β	64a

TABLE I (*Continued*)

Substance	*Source*	*Method of isolation*[a]	*Configuration at biose link*	*References*
3,4,6-Tri-*O*-methyl-D-mannose-(2 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Damson gum	B	β	65
	Cherry gum	B	β	66
D-Xylose-(2 → 1) 4-*O*-methyl-D-glucosiduronic acid	Aspen wood	A	α	67
				See 70d, 70e
3,4-Di-*O*-methyl-D-xylose-(2 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Aspen wood	B	α	67
	Corn-cob hemicellulose	B	α	67a
2-*O*-Methyl-D-xylose-(3 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Pear, cell-wall xylan	C	α	68
	Wheat-straw hemicellulose	C	—	69
2,4-Di-*O*-methyl-D-xylose-(3 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Wheat-straw hemicellulose	B	α	70
	Synthesis	B	β	70
2,3-Di-*O*-methyl-D-xylose-(4 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Corn-cob hemicellulose	B	α	67a
3,4-Di-*O*-methyl-L-rhamnose-(2 → 1) 2,3,4-tri-*O*-methyl-D-galactosiduronic acid	Okra mucilage	B	—	70a
	Berry juice of *Vitis vinifera L.*	B	—	70b
2,3,4-Tri-*O*-methyl-D-galactose-(6 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	*Acacia pycnantha* gum	B	β	70c
D-Xylose-(2 → 1) 4-*O*-methyl-D-glucosiduronic acid	Corn-cob hemicellulose	A	α	70d
	Scots Pine wood	A	α	70e
	Black Spruce wood	A	α	70e
3-*O*-Methyl-D-xylose-(2 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid	Beech wood	C	α	70f
L-Arabinose-(4 → 1) 4-*O*-methyl-D-glucosiduronic acid	Lemon gum	A	α	70g

[a] A, Hydrolysis of naturally-occurring polysaccharide; B, methylation of aldobiouronic acid isolated after graded hydrolysis of polysaccharide; C, hydrolysis of methylated polysaccharide; D, methylation of partially methylated aldobiouronic acid isolated by method C.

(50) E. L. Hirst and Sonia Dunstan, *J. Chem. Soc.*, 2332 (1953).
(51) R. A. Laidlaw and E. G. V. Percival, *J. Chem. Soc.*, 1600 (1949).
(52) E. L. Hirst, E. G. V. Percival and Clare B. Wylam, *J. Chem. Soc.*, 189 (1954).
(53) E. V. White, *J. Am. Chem. Soc.*, **69**, 2264 (1947).
(54) J. I. Cuneen and F. Smith, *J. Chem. Soc.*, 1141 (1948).

pound has been impossible, as no methyl ether of an aldobiouronic acid has yet been obtained crystalline, and only in a few cases have any crystalline derivatives (usually the amide or methyl ester of a methyl glycoside) been isolated. Determinations of structure have normally involved identification of the acidic and non-acidic fragments produced on hydrolysis. Only in the case of the aldobiouronic acid from gum arabic has the configuration at the glycosidic link been conclusively determined,[58,59] although in other cases the optical rotations of the aldobiouronic acids or their derivatives have provided definite indications of the configuration.

Like the parent compounds, the methyl ethers of aldobiouronic acids are resistant to acid hydrolysis, and it is difficult to carry out hydrolysis without some decomposition of the product. This difficulty has recently been overcome by reduction of the uronic acid residue with lithium aluminum hydride[56,67]; the resulting disaccharide then undergoes hydrolysis without difficulty. The first reduction of the uronic acid residue of a methylated aldobiouronic acid methyl ester was accomplished by Levene, Meyer and Kuna,[59] who reduced the methylated aldobiouronic acid from gum arabic with hydrogen in the presence of copper chromite catalyst under the conditions previously used[70h] for reducing the acety-

(55) J. J. Connell, Ruth M. Hainsworth, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1696 (1950).
(56) M. Abdel-Akher, F. Smith and D. Spriestersbach, *J. Chem. Soc.*, 3637 (1952).
(57) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 120 (1948).
(58) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **125,** 355 (1938).
(59) P. A. Levene, G. M. Meyer and M. Kuna, *J. Biol. Chem.*, **125,** 703 (1938).
(60) J. Jackson and F. Smith, *J. Chem. Soc.*, 74 (1940).
(61) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1064 (1947).
(62) F. Brown, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1677 (1948).
(63) J. K. N. Jones, *J. Chem. Soc.*, 534 (1950).
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lated methyl glycoside methyl ester of the same aldobiouronic acid. They showed that, on further methylation, the reduction product gave a compound identical with synthetic methyl 6-*O*-(2,3,4-tri-*O*-methyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-methyl-D-galactoside prepared by Levene and Tipson.[58]

VI. Tables of Properties of Methyl Ethers of Hexuronic Acids and Their Derivatives

The following Tables (II, III, and IV) record appropriate data and references relating to the methyl ethers of some hexuronic acids and their derivatives. In the case of methyl ethers of aldobiouronic acids (Table V), data have been recorded only for crystalline derivatives. Where uronic acids have been characterized by reduction to the corresponding hexose, reference should be made to the previous articles in this series dealing with the methyl ethers of D-glucose,[71] D-galactose,[72] and D-mannose.[73]

Table II

The Methyl Ethers of D-*Glucuronic Acid*

Compound	*Melting point, °C.*	$[\alpha]_D$, *degrees*	*Rotation solvent*	*References*
3-*O*-Methyl-D-glucuronic acid	sirup	+6	H_2O	4
p-bromophenylhydrazine salt of *p*-bromophenylosazone	157	−104 → −14 (equil.)	C_5H_5N	11
4-*O*-Methyl-D-glucuronic acid	sirup	+45	H_2O	6
		+83	H_2O	14
methyl α-glycoside methyl ester	sirup	+128	H_2O	13
amide	236	+150	H_2O	13
methyl β-glycoside amide	232	−50	H_2O	13
2,3-Di-*O*-methyl-D-glucuronic acid	sirup	+42	H_2O	16
		+36	H_2O	17
methyl pyranoside 4-*p*-nitrobenzoate, methyl ester	157	—	—	18
phenylhydrazide	225–227	—	—	18
2,3-Di-*O*-methyl-D-glucaric acid 6-methyl ester 1,4-lactone	101	+14 → +27.7 (10 days)	H_2O	18
diamide	156	+28	H_2O	18
2,5-Di-*O*-methyl-D-glucuronic acid				

(71) E. J. Bourne and S. Peat, *Advances in Carbohydrate Chem.*, **5**, 145 (1950).
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TABLE II (*Continued*)

Compound	*Melting point, °C.*	$[\alpha]_D$, *degrees*	*Rotation solvent*	*References*
methyl α-glycoside 6,3-lactone	131–132	+197.5	H_2O	21
	129–130	+151	$CHCl_3$	22
	132–133	+179 → +134 (139 days)	H_2O	23
amide	121	+149.5	H_2O	23
methyl β-glycoside 6,3-lactone	90–91	+2.0	H_2O	22
		−2.3	$CHCl_3$	22
amide	95	—	—	24
2,5-Di-*O*-methyl-D-glucaric acid 1-methyl ester 6,3-lactone	sirup	+56	H_2O	23
diamide	175	+17	H_2O	23
3,4-Di-*O*-methyl-D-glucuronic acid	184	+60 (equil.)	H_2O	24a
methyl glycoside, amide	191–193	+100	H_2O	9
2,3,4-Tri-*O*-methyl-D-glucuronic acid	sirup	+58 (equil.)	H_2O	25
		+45	H_2O	74
methyl α-glycoside methyl ester	sirup	156	CH_3OH	13
amide	183	+137.5	H_2O	74
	188–189	+149	H_2O	13
methyl β-glycoside	133	−38	H_2O	25
	137	—	—	28
methyl ester	sirup	—	—	74
amide	193	−47	H_2O	74
2,3,4-Tri-*O*-methyl-D-glucaric acid, 6-methyl ester, 1,5-lactone	106.4	+175.9	C_6H_6	75
	107	+103 → +32 (equil.)	H_2O	74
		+102 → +52 (equil.)	CH_3OH	74
	110	+102	C_2H_5OH	65
	112	—	—	76
2,3,5-Tri-*O*-methyl-D-glucuronic acid, methyl α-glycoside, methyl ester	sirup	+122	H_2O	23
2,3,5-Tri-*O*-methyl-D-glucaric acid 6-methyl ester, 1,4-lactone	78	−10	H_2O	23
	78	−9.5 → +6 (102 days)	H_2O	77
diamide	213	+18	H_2O	77

(74) F. Smith, *J. Chem. Soc.*, 1724 (1939).
(75) J. Pryde and R. T. Williams, *Biochem. J.* (London), **27**, 1197 (1933).
(76) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1482 (1939).
(77) F. Smith, *J. Chem. Soc.*, 571 (1944).

TABLE III
The Methyl Ethers of D-*Galacturonic Acid*

Compound	*Melting point, °C.*	$[\alpha]_D$, *degrees*	*Rotation solvent*	*References*
2-*O*-Methyl-D-galacturonic acid, methyl α-D-pyranoside				
methyl ester	sirup	+80	H_2O	30
amide	174	+55	C_2H_5OH	30
	174–175	+60	H_2O	31
2-*O*-Methylgalactaric acid	200	—	—	30
diamide	205	—	—	31
2,3-Di-*O*-methyl-D-galacturonic acid	sirup	+62	H_2O	37
methyl β-D-furanoside				
methyl ester	sirup	−64	H_2O	34
amide	124	−151	H_2O	34
methyl β-D-pyranoside				
methyl ester	111	−11	H_2O	35
2,3-Di-*O*-methylgalactaric acid, 6-methyl ester 1,4-lactone	92	−56 → −4 (29 days, equil.)	H_2O	34
	96	−40	H_2O	36
diamide	228	—	—	34
bismethylamide	184	—	—	34
2,4-Di-*O*-methyl-D-galacturonic acid	sirup	+93	H_2O	39
3,4-Di-*O*-methyl-D-galacturonic acid	sirup	+37	C_2H_5OH	31
		+93	H_2O	31
methyl α-glycoside	154–155	+158	$CHCl_3$	31
		+156	CH_3OH	31
		+163	H_2O	31
methyl ester	113–114	+165	$CHCl_3$	31
amide	130–131	+108	C_2H_5OH	31
methylamide	205	+116	H_2O	31
3,4-Di-*O*-methylgalactaric acid				
diethyl ester	148–149	0	H_2O	31
dimethyl ester	172–173	0	H_2O	31
diamide	230	0	H_2O	31
2,3,4-Tri-*O*-methyl-D-galacturonic acid, monohydrate	96–98	+126.3 → 104.2 (1 hr., equil.)	H_2O	41
	98–99	+120 → +104	H_2O	39
methyl α-glycoside, methyl ester	70	+142.1	$CHCl_3$	40
	70	+149.3	$(CH_3)_2CO$	42
	73	+169	H_2O	28
amide	153–153.5	+121.5	$CHCl_3$	78
	154	+139	H_2O	35

TABLE III (*Continued*)

Compound	*Melting point, °C.*	$[\alpha]_D$, *degrees*	*Rotation solvent*	*References*
methyl β-glycoside	102	−21	CH_3OH	35
		−7	H_2O	35
2,3,4-Tri-*O*-methylgalactaric acid	100–101	+42.0	$(CH_3)_2CO$	40
dimethyl ester	101.5	+29.0	H_2O	40, 51
diamide	273(d)	—	—	74
bismethylamide	205	+7.5	H_2O	74
	207	+12.6	CH_3OH	40
2,3,5-Tri-*O*-methyl-D-galacturonic acid, methyl β-glycoside				
methyl ester	43	−129	CH_3OH	38
amide	106	−151.5	H_2O	34
2,3,5-Tri-*O*-methylgalactaric acid				
6-methyl ester 1,4-lactone	62	−8[3]	H_2O	34
diamide	255	—	—	34
bismethylamide	232	−22	H_2O	38

TABLE IV

The Methyl Ethers of D-*Mannuronic Acid*

Compound	*Melting point, °C*	$[\alpha]_D$, *degrees*	*Rotation solvent*	*References*
2,3-Di-*O*-methyl-D-mannuronic acid	sirup	+30	CH_3OH	45
2,3,4-Tri-*O*-methyl-D-mannuronic acid	sirup	+36.4	H_2O	45
methyl α-glycoside methyl ester	sirup	+74	CH_3OH	47
2,3,4-Tri-*O*-methyl-D-mannaric acid, diamide	228	−17	CH_3OH	45, 49

(78) P. A. Levene and L. C. Kreider, *J. Biol. Chem.*, **121**, 155 (1937).

TABLE V

The Methyl Ethers of Aldobiouronic Acids

Compound	*Melting point, °C*	$[\alpha]_D$, *degrees*	*Rotation solvent*	*References*
(Methyl 3,4-di-*O*-methyl-L-rhamnoside)-(2 → 1) 2,3,4-tri-*O*-methyl-D-galactosiduronic acid, methyl ester	93–94	+129.8	H_2O	43
(Methyl 2,4-di-*O*-methyl-D-galactoside)-(3 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid, amide	194	—	—	53
(Methyl 2,4,6-tri-*O*-methyl-D-galactoside)-(3 → 1) 2,3,4-tri-*O*-methyl-D-glucosiduronic acid, amide	156	—	—	53
(Methyl α-D-galactoside)-(6 → 1) 4-*O*-methyl-β-D-glucosiduronic acid, amide	267	+25	H_2O	13, 56
(Methyl 2,3,4-tri-*O*-methyl-α-D-galactoside)-(6 → 1) 2,3,4-tri-*O*-methyl-β-D-glucosiduronic acid, amide	160	+40.5	H_2O	56
(Methyl 2,3,4-tri-*O*-methyl-β-D-galactoside)-(6 → 1) 2,3,4-tri-*O*-methyl-β-D-glucosiduronic acid				
methyl ester	86	−21	H_2O	25
		−43	$CHCl_3$	25
	94	−21	H_2O	60
amide	196	−18	H_2O	60
(Methyl 2,3,6-tri-*O*-methyl-β-D-glucoside)-(4 → 1) 2,3,4-tri-*O*-methyl-β-D-glucosiduronic acid, methyl ester	171.5–172	−32.6	(H_2O)	64a

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The Alkali-soluble Polysaccharides of the Lichen Cladonia alpestris *(Reindeer Moss).*

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A preliminary examination has shown that the alkali-soluble polysaccharides of reindeer moss (*Cladonia alpestris*) consist of mixtures of highly branched molecules containing residues of D-galactose, D-glucose, and D-mannose. Methylation studies have shown that the majority of the D-galactose and some of the D-glucose and D-mannose residues occupy terminal positions, while chains of D-glucose and of D-mannose residues constitute the backbone of the molecular structure. Periodate oxidation provides further evidence for complex highly branched structures.

The lichen *Cladonia alpestris*, commonly known as reindeer moss, is one of three closely related species, *C. alpestris*, *C. rangiferina*, and *C. sylvatica*, of common occurrence in Norway. Little is known concerning their composition but Professor Berner and his colleagues at Oslo University showed that reindeer moss, which contains 93% of carbohydrates, gives glucose, galactose, and mannose on hydrolysis (personal communication). This lichen appeared to differ considerably from Iceland moss (*Cetraria islandica*), the polysaccharides of which have been extensively investigated and have been shown to consist predominantly of D-glucose residues (Granichstädten and Percival, *J.*, 1943, 54; Meyer and Gürtler, *Helv. Chim. Acta*, 1947, **30**, 751, 761; Chanda and Hirst, unpublished work), although small quantities of D-galactose and D-mannose have been reported in the hydrolysates of some polysaccharide fractions. In the present investigation the alkali-soluble polysaccharides of *C. alpestris* have been examined, preliminary experiments having shown that after removal of lichen acids with aqueous sodium carbonate very little polysaccharide could be extracted with hot water.

Two polysaccharide preparations (I and II) were obtained by extraction of the lichen with cold 5% and 24% potassium hydroxide solution respectively. The polysaccharides, although differing in optical rotation, had similar physical properties and gave the same sugars (galactose, glucose, and mannose) on hydrolysis, but in different proportions. D-Galactose and D-mannose were identified in both hydrolysates by the formation of crystalline derivatives. Fractionation of polysaccharide I *via* the copper complex gave a polysaccharide of different composition, indicating the presence of at least two different molecular species, but repeated fractionation failed to resolve the mixture.

Conversion of each of the two polysaccharides into their fully methylated derivatives gave similar products, differing slightly in optical rotation, but giving on hydrolysis the same complex mixture of methylated sugars. The methylated polysaccharides were therefore combined for subsequent examination. The methylated sugars (fraction A) obtained on hydrolysis of the methylated polysaccharides were fractionated on cellulose, but in most of the fractions separation of the sugars was incomplete. Although 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose was the only sugar from fraction A to be identified by the formation of a crystalline derivative, evidence from optical rotations, paper chromatography, and demethylation indicated the presence also of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose, a mixture of tri-*O*-methyl derivatives of D-glucose and D-mannose including 2 : 3 : 6-tri-*O*-methyl-D-glucose, 2 : 4 : 6-tri-*O*-methyl-D-galactose, and a di-*O*-methyl-D-glucose (probably the 2 : 3-isomer). In addition to these products of hydrolysis two portions (B and C) resistant to acid hydrolysis were also encountered, one of them (C) coming out of solution during the treatment with aqueous acid. These fragments were hydrolysed under more vigorous conditions and the products were further fractionated. Fraction B was shown to be an incompletely methylated fragment composed of residues of D-mannose only. From the hydrolysis products 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose was characterised as the

crystalline aniline derivative and 3 : 4-di-*O*-methyl-D-mannose was identified as the crystalline monohydrate; in addition, paper chromatography indicated the presence of a tri-*O*-methyl-D-mannose, a second di-*O*-methyl-D-mannose, and a mono-*O*-methyl-D-mannose. Fraction C was shown to be composed solely of D-glucose residues and in the mixture of methylated sugars given on hydrolysis 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose was identified.

The yield of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose indicates that the majority of D-galactose residues are present as non-reducing end-groups, while some of the D-glucose and D-mannose residues also occupy terminal positions. The isolation of the resistant fragments B and C indicates the presence of two main structural features, which are remarkable in this group of polysaccharides in that each consists of chains of one type of sugar residue only. It is not possible on the present evidence to decide whether D-galactose residues are linked to both types of molecular structure or only to one.

The results of periodate oxidation experiments are consistent with the view that polysaccharides I and II both contain mixtures of highly branched polysaccharides. Oxidation of the polysaccharides resulted in the formation of 1 mol. of formic acid per 3·7 and 3·0 hexose residues respectively, while both polysaccharides consumed 1·2 mols. of periodate per residue. Confirmation was thus obtained for the presence of a high proportion of non-reducing end-groups. Hydrolysis of the periodate-oxidised polysaccharides indicated the presence of unattacked glucose residues and also in smaller amount of unattacked mannose residues. The former observation suggests the presence in the polysaccharides of glucose residues linked through positions 1 and 3, in which case it seems probable that 2 : 4 : 6-tri-*O*-methyl-D-glucose was one component of the mixture of incompletely identified tri-*O*-methylhexoses isolated from the methylated polysaccharide hydrolysate. The latter observation would be expected if the 3 : 4-di-*O*-methyl-D-mannose, previously isolated, has structural significance and does not arise from incomplete methylation. It is interesting that a trace of galactose was also given on hydrolysis of the oxidised polysaccharide I, suggesting the presence of a small proportion of D-galactose residues linked through positions 1 and 3. This provides further evidence that the tri-*O*-methyl-D-galactose present among the methylated sugars was indeed the 2 : 4 : 6-isomer.

It is evident from these preliminary experiments that the alkali-soluble polysaccharides of reindeer moss contain highly branched complex structures. The results emphasize the necessity of applying new methods of fractionation before the number and detailed structure of the components present in this lichen can be established.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems: (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3; v/v; top layer) and (B) butan-1-ol–ethanol–water (4 : 1 : 5; v/v; top layer).

Isolation of Alkali-soluble Polysaccharides from Cladonia alpestris.—Extractive-free lichen was extracted successively with cold dilute sodium carbonate solution and boiling water to remove lichen acids and water-soluble polysaccharides, and the residue was extracted with cold 5% and 24% potassium hydroxide solutions. The alkaline extracts were poured into ethanol acidified with glacial acetic acid and the precipitated polysaccharides (I and II respectively) were dried by solvent exchange with ethanol and ether. Polysaccharide I, isolated in 2·2% yield, had $(\alpha)_D^{15}$ +43·9° (*c*, 0·4 in 2N-NaOH) and chromatographic examination of the hydrolysate (Flood, Hirst, and Jones, *J.*, 1948, 1679) in solvent (A) showed the presence of galactose (12·6%), glucose (51·7%), and mannose (34·2%). Polysaccharide II, isolated in 1·3% yield, had $[\alpha]_D^{15}$ +61·4° (*c*, 0·89 in 2N-NaOH) and chromatographic examination of the hydrolysate showed the presence of galactose (12·9%), glucose (39·7%), and mannose (47·4%). D-Galactose and D-mannose were identified in both hydrolysates by the formation of the methylphenylhydrazone (m. p. and mixed m. p. 176—178°) and the phenylhydrazone (m. p. and mixed m. p. 198—199°) respectively.

A sample of polysaccharide I was fractionated by precipitation of the copper complex formed on addition of Fehling's solution to a solution of the polysaccharide in aqueous sodium hydroxide, followed by decomposition of the copper complex with dilute hydrochloric acid and precipitation of the regenerated polysaccharide with ethanol. The regenerated polysaccharide gave on hydrolysis galactose (15·2%), glucose (28·2%), and mannose (49·8%). These results showed that polysaccharide I was inhomogeneous, but repeated fractionations *via* the copper complex failed to yield distinct components.

Methylation of Polysaccharides.—Polysaccharides I (6·6 g.) and II (3·8 g.) were each methylated fifteen times with methyl sulphate and sodium hydroxide solution and twice with methyl iodide and silver oxide. Methylated polysaccharide I had $[\alpha]_D^{17}$ +35° (*c*, 0·5 in $CHCl_3$) (OMe, 44·5%) and methylated polysaccharide II had $[\alpha]_D^{17}$ +24·5° (*c*, 0·6 in $CHCl_3$) (OMe, 44·0%). Samples of both methylated polysaccharides were hydrolysed and the hydrolysates were examined chromatographically by use of solvent (B) and shown to contain qualitatively similar mixtures of methylated sugars.

Hydrolysis of Methylated Polysaccharides and Separation of Methylated Sugars.—The mixture of methylated polysaccharides I (1·3 g.) and II (2·7 g.) was refluxed with methanolic 1% hydrogen chloride (400 c.c.) for 17 hr. An insoluble residue (0·1 g.) was separated, and the hydrolysate was neutralised with ethereal diazomethane and concentrated to a syrup. The syrup was hydrolysed on the water bath with N-hydrochloric acid (200 c.c.) for 8·5 hr., during which a flocculent solid (soluble in methanol) separated. The solid was re-treated with methanolic and aqueous hydrogen chloride but a residue (C), insoluble in hydrochloric acid, remained and was separated. The combined acid hydrolysates were neutralised with silver carbonate and concentrated to a syrup (A) (3·2 g.).

Syrup A was fractionated on cellulose (Hough, Jones, and Wadman, *J.*, 1949, 2511) with light petroleum–butan-1-ol (7 : 3), saturated with water, as eluant to give five main fractions (see Table) together with a number of smaller fractions (combined wt., 48 mg.) which were only examined chromatographically and shown to contain mixtures of di- and mono-*O*-methylsugars. Elution of the cellulose with water gave a non-reducing syrup (B) (0·64 g.) which was examined separately. Examination of fraction AI showed it to contain only 32% of reducing sugar (hypoiodite oxidation) and demethylation (Hough, Jones, and Wadman, *J.*, 1950, 1702) showed that methyl ethers of galactose, glucose, and mannose were present. The fraction (0·37 g.) was rehydrolysed and part of the hydrolysate (0·20 g.) was separated on filter sheets (Whatman 3MM) by use of solvent B to give four fractions.

Fraction	Wt. of material eluted (mg.)	$[\alpha]_D^{17}$ (solvent) *	Found : OMe (%)	Calc. : OMe (%)	Paper chromatography R_G in solvent B	Sugar
AI	799	+49·7° (W)	45·0	—	0·95	—
AI*a*	40	+23 (W)	49·0	52·5	1·00	Tetra-*O*-methylmannose
AI*b*	32	+53 (W)	46·1	—	1·00 0·93 0·83	,, Tetra-*O*-methylgalactose Tri-*O*-methylhexose
AI*e*	52	+22·4 (W)	43·7	41·9	0·83	,,
AI*d*	6	—	—	—	0·63	Di-*O*-methylhexose
AII	333	+109 (W) +76·3 (E)	50·1	52·5	0·91	Tetra-*O*-methylgalactose
AIII	483	+56·5 (W)	39·8	41·9	0·84	Tri-*O*-methylhexose
AIV	132	+80·4 (W)	36·7	—	0·71 0·65	Tri-*O*-methylgalactose Di-*O*-methylglucose
AV	66	+55·3 (W)	29·6	29·8	0·65	,,

* W = H_2O, E = EtOH.

Fraction AI*a* travelled on the chromatogram at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose and on demethylation gave mannose. Quantitative paper chromatography (Hirst, Hough, and Jones, *J.*, 1949, 298) showed that tetra- and tri-*O*-methylhexoses were present in fraction AI*b* in the ratio 2 : 1. The presence of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose in this fraction was shown by conversion into the aniline derivative, m. p. and mixed m. p. 186—189°. Demethylation of fraction AI*e* gave mannose and glucose, indicating the presence of trimethyl ethers of both these sugars. Fraction AII was chromatographically homogeneous and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 189—191° (Found : OMe, 39·6. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·9%). The presence of trimethyl ethers of glucose and mannose in fraction AIII was shown by demethylation. The fall in rotation of the fraction in methanolic 1% hydrogen chloride at room temperature, $[\alpha]_D^{17}$ +50° → +27° (*c*, 0·2), indicated that 2 : 3 : 6-tri-*O*-methyl-D-glucose was one component of the mixture. {Under similar conditions 2 : 3 : 6-tri-*O*-methyl-D-glucose showed $[\alpha]_D$ +70° → −37° and 2 : 3 : 6-tri-*O*-methyl-D-mannose showed $[\alpha]_D^{17}$ +11° (const.)}. A sample of fraction AIII was oxidised with sodium metaperiodate but the absence of formaldehyde as shown by the Rimini ferricyanide–phenylhydrazine test (*Bull. Soc. chim.*, 1898, **20**, 896) indicated the absence of 2 : 3 : 4-tri-*O*-methylhexose. The presence in fraction AIII of a small quantity of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, which had escaped chromatographic detection, was shown by formation of the aniline derivative, m. p. and mixed m. p. 185—187° : no

other crystalline aniline derivatives were isolated. Fraction AIV contained two sugars travelling on the chromatogram at the same rate as 2 : 4 : 6-tri-*O*-methyl-D-galactose and 2 : 3-di-*O*-methyl-D-glucose and gave on demethylation galactose and glucose. Quantitative paper chromatography and calculation from the optical rotations showed that the relative proportion of the two sugars was 7 : 3. Fraction AV was chromatographically homogeneous, travelling at the same rate as 2 : 3-di-*O*-methyl-D-glucose, and gave glucose on demethylation.

Examination of Non-reducing Syrup B.—Syrup B had $[\alpha]_D^{17}$ +30° (*c*, 0·5 in H_2O) (Found : OMe, 31·8%) and on demethylation gave only mannose. A portion (0·45 g.) was hydrolysed on the water-bath successively with formic acid (45 c.c. ; 95%) for 6·5 hr. and with N-sulphuric acid (30 c.c.) for 3 hr. The hydrolysate was neutralised with barium carbonate, and concentrated to a syrup (0·381 g.), part of which (0·320 g.) was fractionated on cellulose to give five main fractions BI—V. A number of smaller fractions (combined wt. 66 mg.) and a resistant residue (119 mg.), eluted with water, were not examined further.

Fraction BI (41·4 mg.) had $[\alpha]_D^{17}$ +21·7° (*c*, 0·8 in $CHCl_3$) and travelled on the chromatogram at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose. It was identified by conversion into 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-mannosylamine, m. p. and mixed m. p. 143—145°. Fraction BII (30 mg.) had $[\alpha]_D^{17}$ +14·5° (*c*, 0·6 in $CHCl_3$) and $[\alpha]_D^{17}$ +8·7° (*c*, 0·5 in H_2O), and travelled on the chromatogram at the same rate as 2 : 3 : 6-tri-*O*-methyl-D-mannose. Fraction BIII (30 mg.) crystallised and after recrystallisation from ethanol had m. p. and mixed m. p. (with authentic 3 : 4-di-*O*-methyl-D-mannose monohydrate) 78—80° and $[\alpha]_D^{17}$ +5° (*c*, 0·6 in H_2O ; equil.). The two samples gave identical *X*-ray crystal photographs (by the courtesy of Dr. C. A. Beevers) and it is concluded that the authentic specimen, which originally had m. p. 109° (Haworth, Hirst, and Isherwood, *J.*, 1937, 784), had changed to a more stable crystalline form with m. p. 80—82°. Fraction BIV (20 mg.) had $[\alpha]_D^{17}$ +7·5° (*c*, 0·4 in acetone) and R_G 0·62 in solvent B (3 : 4-di-*O*-methyl-D-mannose had R_G 0·65). Fraction BV (27 mg.) had $[\alpha]_D^{17}$ −5·3° (*c*, 0·3 in H_2O) and R_G 0·32 in solvent B, corresponding to a mono-*O*-methyl-D-mannose.

Examination of Residue C.—The solid C (0·5 g.) was hydrolysed on the water-bath successively with formic acid (10 c.c. ; 95%) for 7 hr. and with N-sulphuric acid (3 c.c.) for 6 hr., considerable decomposition occurring. After neutralisation with barium carbonate, the hydrolysate was concentrated to a syrup (0·250 g.), demethylation of which gave glucose only. A portion of the syrup (110 mg.) was separated on filter sheets with solvent B to give four fractions.

Fraction CI (30 mg.) crystallised and after recrystallisation from ether had $[\alpha]_D^{17}$ +80° (*c*, 0·3 in H_2O) and m. p. and mixed m. p. (with authentic 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose) 86—88°. Fraction CII (12 mg.) had $[\alpha]_D^{17}$ +68° (*c*, 0·2 in acetone) and R_G 0·86 in solvent B. The rotation of the fraction in methanolic 1% hydrogen chloride, $[\alpha]_D^{17}$ +33° (*c*, 0·2), indicated that 2 : 3 : 6-tri-*O*-methyl-D-glucose was one component of a mixture of tri-*O*-methyl-D-glucoses. {All other tri-*O*-methyl-D-glucopyranoses show $[\alpha]_D$ *ca.* +70° in methanolic hydrogen chloride (Granichstädten and Percival, *loc. cit.*).} Fraction CIII (35 mg.) travelled on the chromatogram at the same rate as 2 : 3-di-*O*-methyl-D-glucose but the optical rotation {$[\alpha]_D^{17}$ = +76° (*c*, 0·7 in acetone)} was higher than that quoted for 2 : 3-di-*O*-methyl-α-D-glucose {$[\alpha]_D^{17}$ = +81·9° → +48·3° in acetone (Irvine and Scott, *J.*, 1913, **103**, 575)}. Fraction CIV (16 mg.) had R_G 0·34 in solvent B, corresponding to a mono-*O*-methyl-D-glucose, but was not examined further.

Periodate Oxidation of Polysaccharides.—Oxidation of polysaccharide I (50-mg. batches) with potassium metaperiodate solution by the method of Halsall, Hirst, and Jones (*J.*, 1947, 1399, 1427) yielded a constant amount of formic acid after 208 hr., corresponding to 1 mol. per 3·7 $C_6H_{10}O_5$ residues. Oxidation of polysaccharide II yielded formic acid corresponding to 1 mol. per 3·0 $C_6H_{10}O_5$ residues.

Oxidation of the polysaccharides with sodium metaperiodate solution showed that both polysaccharides consumed 1·2 mols. of periodate per $C_6H_{10}O_5$ residue. Chromatographic examination of the hydrolysates of the periodate-oxidised polysaccharides showed the presence of glucose and mannose (trace), and from polysaccharide I galactose (trace) also.

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The Extracellular Polysaccharide of *Aerobacter Aerogenes* A3 (S1) (Klebsiella Type 54)

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Although it has long been known that organisms of the Aerobacter–Klebsiella group produce very mucoid growths, few attempts have been made to investigate the nature of their capsules and slime. Recently, attention has been drawn to the extracellular polysaccharides of these organisms by the work of Edwards & Fife (1952), who showed that these bacteria exist in a large number of immunologically distinct types. Edwards & Fife identified fifty-seven types, but this has since been increased to nearly 100 (Edmunds, 1954; Henriksen, 1954). These extracellular polysaccharides may occur as loose slime or as distinct capsules and the amount produced varies considerably according to the nature of the growth medium (Duguid & Wilkinson, 1953; Wilkinson, Duguid & Edmunds, 1954). They are also responsible for the distinctive antigenic specificity of strains in the mucoid phase (Wilkinson *et al.* 1954).

Chemical studies on the nature of *Aerobacter aerogenes* extracellular polysaccharides have been carried out on untyped strains. Schardinger (1902*a*, *b*) isolated a galactan from a 6-week broth culture of *Aero. aerogenes* but the product may have been an intracellular polysaccharide. Tomcsik (1927) isolated a polysaccharide by treatment of capsulated cells with potassium hydroxide, and Warren (1950) obtained a slime fraction, but neither author identified the component sugars.

No attempts have been made to determine the influence of different carbon sources on the relative amounts of the component sugars in a heteropolysaccharide. Morgan & Beckwith (1939) cultured a strain of *Escherichia coli* on media containing lactose, glucose, rhamnose or xylose as the carbon source and showed that the immunological specificity of the strain was unaffected. However, this may only mean that a certain immunologically determinant group is present in the extracellular polysaccharide and the overall composition may have varied. Forsyth & Webley (1949) grew various species of the genus *Bacillus* on media containing lactose, fructose, glucose, galactose or arabinose, and found that the component sugars of the extracellular polysaccharide remained unaltered although quantitative determinations were carried out.

In the present study, the composition of the extracellular slime polysaccharide of *Aero. aerogenes* strain A3 (S1) (Klebsiella Type 54) has been studied after growth on media containing different carbon sources. The constituent sugars have been identified and the different polysaccharides compared.

EXPERIMENTAL AND RESULTS

Growth of organism

Organism. *Aero. aerogenes* A3 (S1) is a non-capsulate organism producing an abundance of extracellular polysaccharide as a viscous slime. Immunologically it belongs to Type 54 of the Edwards classification, and its origin and biochemical characteristics are described by Wilkinson *et al.* (1954).

Growth medium. The organism was grown in a medium containing a relatively high sugar and low nitrogen-source concentration which gives maximal polysaccharide production (Wilkinson *et al.* 1954). Growth was carried out in 1 l. screw-capped round bottles, each containing 200 ml. of the following medium: 1·0% Na_2HPO_4; 0·3% KH_2PO_4; 0·03% $(NH_4)_2SO_4$; 0·1% K_2SO_4; 0·1% NaCl; 0·02% $MgSO_4, 7H_2O$; 0·002% $CaCl_2, 6H_2O$; 0·0001% $FeSO_4$; 1% carbohydrate (glucose, sucrose, galactose, mannitol, xylose, rhamnose, fucose or glucurone; in the case of glucurone, sufficient NaOH was added to adjust the pH to 7·3). The medium was sterilized by steaming and inoculated from a 24 hr. culture previously grown and adapted to the sugar to be used in the growth medium. The bottles were then filled with O_2 through sterile plugged tubing for 2 min. In order to maintain adequate oxygenation of the medium during growth, the bottles were rotated horizontally on a drum of horizontal axis capable of holding six bottles at a time and mounted in a 35° incubator. The cultures were grown for 48 hr.

General analytical methods

Total nitrogen was determined by the micro-Kjeldahl method. Total phosphorus was determined by the Fiske & Subbarow (1925) method. The ash content was determined by incinerating a little of the material in a Pt boat to constant weight, a drop of 60% (w/v) $HClO_4$ being added after the first ignition.

The reducing value of the unhydrolysed and hydrolysed polysaccharides was determined using Somogyi's (1945) copper reagent in conjunction with a colorimetric reagent (Nelson, 1944) calibrated against glucose. The results were expressed in terms of percentage glucose.

The anthrone value. The modified anthrone method of Fairbairn (1953) was adopted. The method, which is finding increasing application as a rapid and sensitive means of estimating polysaccharides, is of little direct value in composition studies. However, it was found to be useful as an independent check on the composition data derived by the methods described below. This was done by comparing the experimental anthrone value of a polysaccharide with the anthrone value calculated from the composition data. The 'anthrone equivalents' of the component sugars (colour given by 100 μg. of the sugar/colour given by 100 μg. glucose) were as follows: galactose, 0·60; fucose, 0·92; glucurone, 0·05. All results were expressed in terms of percentage glucose.

Determination of component sugars

Glucose, galactose and fucose. These sugars were estimated after hydrolysis and chromatographic separation on paper according to the general principles described by Flood, Hirst & Jones (1948). The polysaccharides were found to be polyuronides and resistant to the normal methods of hydrolysis such as N-H_2SO_4 at 100° for 24 hr. The conditions finally adopted as the standard method for quantitative studies which gave complete hydrolysis as indicated by a maximal reducing value, were 24 hr. in 96% (w/v) formic acid at 100°, followed by 6 hr. in N-H_2SO_4 at 100°. Samples of polysaccharide (20–40 mg.) were hydrolysed for 24 hr. with 96% formic acid (2 ml.) in a sealed tube in a boiling-water bath. (Preliminary experiments showed that a polysaccharide concentration within the range 10–20 mg./ml. gave minimal destruction.) A weighed quantity (15–30 mg.) of reference sugar (arabinose), shown chromatographically to be absent from the polysaccharide hydrolysate, was added and the formic acid distilled off under reduced pressure. Further hydrolysis was carried out for 6 hr. at 100° after the addition of N-H_2SO_4 (2 ml.). The contents of the tube were neutralized by the addition of $BaCO_3$. It was assumed that the destruction of the reference sugar and the component sugars of the polysaccharide took place at the same rate.

Because of the difficulties introduced by lactone trails on chromatograms and of the anomalous yield of formic acid from periodate oxidation, it was necessary to remove uronic acids from the hydrolysate. Preliminary experiments were made using anion-exchange resins, but these were discontinued because of the possibility that free sugars might be differentially adsorbed on to the resin (Roseman, Abeles & Dorfman, 1952). Finally it was found that the uronic acid could be removed from the neutral hydrolysate without disturbing the ratio of free sugars, by precipitation of the Ba salt. Ethanol (2 vol.) was added to the supernatant after $BaCO_3$ treatment, the tube was left to stand overnight at 4°, and the precipitated Ba salt of the uronic acid was removed by centrifuging. The supernatant was treated with Amberlite cation-exchange resin IR-120 and evaporated to dryness. The syrup was dissolved in an appropriate small volume of water and suitable amounts were applied to the starting line of a strip of Whatman no. 1 filter paper. The solvent used for separation in early experiments was a benzene–butanol–pyridine–water mixture (1:5:3:3, v/v) for 44 hr. at 15°. This gave good separation of sugars but often interfered in the estimations because of erratic high blank values. It was replaced in later experiments by butanol–ethanol–water mixture (5:1:4, v/v), which was found to give equally good separation after 120 hr. at 15° while having reproducible small blank values. After drying, the side strips were sprayed with saturated aqueous aniline oxalate, and the colours developed by heating; it was often found that spots invisible by daylight could be observed under ultraviolet radiation. The sugars were eluted from the corresponding central portion of the chromatogram by the method of Laidlaw & Reid (1950). The amounts of the sugars were estimated by periodate oxidation (Hirst & Jones, 1949).

Fucose. This was also determined separately on the unhydrolysed polysaccharide by the colorimetric method for methylpentoses (Dische & Shettles, 1948). The method was claimed by the authors to be specific for methylpentoses but in trial experiments it was found that all the sugar components of the polysaccharide would affect the result. The relative colour-producing powers of fucose, glucose and glucurone were 1·00, 0·058 and 0·021, respectively, and in each case the colour was proportional to the sugar concentration. Experiments with mixtures of these sugars showed that when the colours produced by glucose and glucurone were subtracted from the total colour, fucose (6–44 μg.) could be estimated in the presence of glucose (68 μg.) and glucurone (37 μg.) with recoveries of 95–112%. By correcting the results for the amounts of glucose and uronic acid (assumed to be glucurone) known to be present in the polysaccharide, the fucose content determined by this method was found to give results comparable to the chromatographic method.

Uronic acid. This was determined on the unhydrolysed polysaccharide by the decarboxylation method of McCready, Swenson & Maclay (1946).

Isolation and purification of the polysaccharide

The culture was centrifuged at 13000 rev./min. for 30 min. and the cellular centrifugate discarded. High-speed centrifuging is necessary because of the viscosity of the slime polysaccharide; the culture supernatant gave a relative viscosity of 22 at 15° as determined in an Ostwald viscometer. Acetone (1·5 vol.) was added to the supernatant and the mixture stirred with a spatula. The polysaccharide gel adhered to the spatula and could be washed and dehydrated by gradually increasing concentrations of acetone until the gel was converted into a stringy white solid. This resulted in a considerable purification of the polysaccharide, since many of the impurities present in the culture supernatant did not adhere to the spatula and were not precipitated by 1·5 vol. acetone (e.g. the extracellular O-polysaccharide). The dehydrated polysaccharide was dissolved to give a 0·5% solution in an acetate buffer containing 4% (w/v) sodium acetate and 2% (v/v) acetic acid. It was then deproteinized by shaking each 100 ml. with six to eight successive lots of a mixture of $CHCl_3$ (20 ml.) and *n*-butanol (4 ml.) (Sevag, 1934). The final aqueous solutions were combined, recentrifuged and precipitated by acetone (1·5 vol.) as described previously. The dried precipitate was redissolved in water to give a 0·2% solution and was dialysed against running tap water for 96 hr. The purified polysaccharide was finally precipitated from solution by one of two methods.

Method A

The solution was shaken alternately with small portions of anion and cation-exchange resins (Amberlite IR-4B and

IR-120). This produced the free acid form of the polysaccharide which was no longer precipitable by acetone. Instead, it was lyophilized.

Method B

Sodium acetate (1%) was added followed by precipitation with acetone (1·5 vol.). The precipitate was washed with acetone and ether and dried in a vacuum desiccator over P_2O_5.

In the procedures described above, it was difficult to remove protein effectively from the polysaccharide preparation because of its high viscosity in solution. It was found that if a solution was boiled at a neutral pH, the viscosity fell to a low value with a consequent increase in the ease of handling. Consequently, the following method was adopted for later preparations.

Method C

The initial precipitate from the culture supernatant was dissolved in distilled water to give a 0·5% solution, the pH was adjusted to 7·0 and the solution was boiled for 30 min. The further stages of deproteinization and purification were as described in method *B*, except that the polysaccharide required the addition of 3 vol. of acetone followed by a period of 12 hr. at 4° to allow complete precipitation.

The yields of polysaccharide isolated by the three methods were of the order of 1 g./3·6 l. of culture.

Separation and identification of the component sugars of the polysaccharide

Polysaccharide (2 g.) was hydrolysed by heating on a boiling-water bath for 24 hr. in 96% formic acid (200 ml.). After removal of the formic acid by distillation under reduced pressure, the resultant syrup was heated at 100° for 6 hr. in N-H_2SO_4 (100 ml.). The product was neutralized with $BaCO_3$, concentrated to 30 ml. and methanol (60 ml.) was added. The mixture was left for 24 hr. at 4° and the precipitate of the Ba salt of the uronic acid was centrifuged. Chromatographic analysis showed that the supernatant contained glucose, fucose and a small amount of uronic acid. There was also a slight trace of an unknown sugar travelling on a chromatogram faster than fucose. This may have been fucoketose produced by prolonged contact with $BaCO_3$, since fucose treated with $BaCO_3$ for 24 hr. at room temperature produced a spot similar in its chromatographic behaviour. (Rate of movement relative to 2:3:4:6-tetramethyl glucose (R_G) in *n*-butanol–ethanol–water (5:1:4, v/v) was 0·36.) The supernatant was evaporated to a syrup and redissolved in water to 60 ml. The remaining uronic acid was removed by passage through a column of Amberlite IRA-400 resin previously converted into the carbonate form. The eluate (500 ml.) containing glucose and fucose, was concentrated and dried over P_2O_5 to give a syrup (1·5 g.), which was fractionated on a cellulose column at 37° by using as the solvent butanol saturated with water as described by Hough, Jones & Wadman (1949). Every tenth tube was examined for the presence of carbohydrate by the anthrone method and for the nature of the sugar chromatographically. The following results were obtained: tubes 1–54, no carbohydrate; tubes 55–95, fucose; tubes 96–124, fucose and glucose; tubes 125–260, glucose.

Identification of L-*fucose.* The syrup (93 mg.) from tubes 55–95 had $[\alpha]_D^{15} - 69°$ in water (*c*, 2) (literature value $-76°$) and paper chromatography showed the presence of fucose only. The identity of the sugar as L-fucose was confirmed by the formation of L-fucose phenylhydrazone (m.p. and mixed m.p. 156–159°).

Identification of D-*glucose.* Syrup (300 mg.) from tubes 125–245 had $[\alpha]_D^{15} + 50°$ in water (*c*, 2) (literature value, $+52·5°$) and paper chromatography showed the presence of glucose only. The identity of the sugar as D-glucose was confirmed by the formation of β-D-glucose penta-acetate (m.p. and mixed m.p. 124–127°).

Identification of the uronic acid. This will be dealt with in a later communication. The fact that a lactone was formed, indicated that the uronic acid was not galacturonic acid.

Analysis of the extracellular polysaccharide from glucose-grown Aerobacter aerogenes

The basic results of the quantitative analysis of the polysaccharide prepared by methods *A*, *B* and *C* after growth on glucose are shown in Table 1. The main components are glucose, fucose and uronic acid. A small amount of galactose was also present but was most likely due to contamination by some extracellular 0-polysaccharide which is probably a galactan (Dudman & Wilkinson, unpublished results). The colorimetric method for the estimation of fucose was found to give more consistent results than the chromatographic method, possibly because of the rather diffuse nature of the fucose spot on the chromatograms. The anthrone value calculated from the analytical data compares well with the experimental result, showing that all the anthrone-reactive carbohydrates in the polysaccharide had been accounted for. It can be seen that the relative proportions of the main component sugars remain constant irrespective of the mode of preparation, showing that boiling the polysaccharide solution caused no change in composition in spite of the great lowering in viscosity.

Further analysis was carried out on the polysaccharide prepared by method *A*. The specific rotation was $[\alpha]_D^{15} - 50° \pm 5°$ in water (*c*, 0·1). The uronic acid content, determined by the naphthoresorcinol method (Jarrige, 1947), was 22%. It was found over a range of different heteropolysaccharides, that this method gave consistently low values compared with those by decarboxylation, possibly owing to difficulty in obtaining complete hydrolysis of the polysaccharide without decomposition of the uronic acid. The acid equivalent was 760, corresponding to a uronic acid content of 25·5%. The reducing value after hydrolysis was 78%. Amino acids and hexosamines were absent as indicated by negative results after paper chromatography using respectively, ninhydrin and the Elson & Morgan (1933) reagent as developers. Ketoses were absent, as indicated by negative results after paper chromatography using naphthoresorcinol reagent as developer and also by the Seliwanoff reaction on the intact polysaccharide.

Analysis of the extracellular polysaccharide of Aero. aerogenes *after growth on various carbon sources*

The results of analysis of the extracellular polysaccharide produced by *Aero. aerogenes* after growth on a variety of carbohydrates as the sole carbon and energy source and isolated by method *C*, are shown in Table 1. The carbohydrates were chosen to give representative types of sugars as well as those normally occurring in the polysaccharide. The proportions of the component sugars, glucose, fucose and uronic acid, can be seen to be identical within the limits of experimental error in each of the polysaccharides.

It has been reported that *Bacillus polymyxa* when grown on monosaccharides produces an extracellular polyuronide while after growth on sucrose it produces a polyuronide together with an extracellular levan (Hestrin, Avineri-Shapiro & Aschner, 1943; Forsyth & Webley, 1949). Consequently, *Aero. aerogenes* was grown on sucrose as the sole source of carbon and energy, and the polysaccharide isolated according to method *C*. A full analysis was not made but fructose was shown to be absent by paper chromatography after mild acid hydrolysis and by the Seliwanoff reaction on the intact polysaccharide.

DISCUSSION

All extracellular heteropolysaccharides produced by bacteria from widely different species and examined with the aid of modern techniques, have been found to contain uronic acid or hexosamine components. The extracellular polysaccharide of *Aero. aerogenes* A 3 (S 1) is a polyuronide and therefore no exception to this rule. An unusual feature is the presence of L-fucose. Until a recent report by Norris, de Sipin, Zilliken, Harvey & György (1954) that fucose was a component of the polysaccharide of a mucoid mutant of *Lactobacillus bifidus*, fucose has never been found in a bacterial polysaccharide. However, another naturally occurring methylpentose, rhamnose, has been identified in the polysaccharides of a variety of strains (see Whistler & Smart, 1953).

An ultracentrifugal examination of the polysaccharide, prepared by method *A* after growth on glucose, indicated the presence of only one component with a narrow molecular weight distribution, thus suggesting the presence of a single polysaccharide rather than a mixture of two or more molecular species (C. T. Greenwood, 1954; unpublished results).

The amount of the three main components, glucose, fucose and uronic acid, was found to be independent of the carbon source used in the growth medium. This has a bearing on the mode of

Table 1. *Analytical results on the polysaccharide of* Aerobacter aerogenes *after growth on various carbon sources*

	Sugar in growth medium								
	Glucose			Galactose	Mannitol	Xylose	Rhamnose	Fucose	Glucurone
Method of preparation	*A*	*B*	*C*	*C*	*C*	*C*	*C*	*C*	*C*
Glucose (%)	46	46	50	45	43	49	47	44	46
Galactose (%)	2	2	1	1	2	2	2	2	1
Fucose (%)									
(*a*) Chromatographically	10		10	7	4	8	8	7	7
(*b*) Colorimetrically	9		10	9	9	10	9	9	9
Uronic acid (%)	27	28	29	28	25	28	26	27	29
Total % sugar components	84	85	90	83	79	89	84	82	85
Fucose } relative to	20	20	20	20	21	20	19	20	20
Uronic acid } glucose as 100	58	61	58	61	58	58	56	61	62
Reducing value of unhydrolysed polysaccharide	1·8	1·6	0·7	0·8	1·4	1·3	0·8	1·2	1·3
Anthrone value (%)	54	55	64	59	53	60	52	55	57
Anthrone value theoretical (%)	56	56	60	54	53	60	57	55	56
Ash (%)	1·3	4·0	7·6	7·0	6·3	4·5	4·0	3·7	2·9
N (%)	1·05	0·18	0·17	0·15	0·15	0·39	0·79	0·65	0·89
P (%)	0·10	0·12	0·04	0·04	0·06	0·19	0·49	0·45	0·43

synthesis of such heteropolysaccharides. Work on homopolysaccharides summarized by Barker & Bourne (1953) and by Hassid (1954) has shown that they are synthesized according to the general equation:

$$Gt\text{–}O\text{–}X + H\text{–}O\text{–}Gr \rightleftharpoons Gt\text{–}O\text{–}Gr + X\text{–}O\text{–}H,$$

where Gt–O and X are, respectively, the sugar residue and the aglycone portion of a glycoside donor (Gt–O–X) which serves as the substrate for an enzyme, and Gr–O–H is the carbohydrate receptor molecule. Two main types of compounds have been demonstrated as glycoside donors, (1) disaccharides and higher saccharides where X is a carbohydrate residue, (2) sugar phosphates where X is a phosphate residue. However, no evidence has been obtained as to the nature of the glycoside donors in heteropolysaccharide formation although they may be assumed to function in a similar manner. By varying the nature of the carbon source during growth and polysaccharide formation, one can be assumed to vary the equilibrium concentrations of these glycoside donors so that the relative proportions are different. This follows unless all the carbon sources are metabolized to a common intermediate from which all the glycoside donors are formed. If polysaccharide synthesis is carried out by a comparatively non-selective enzyme or enzymes which string together glycoside radicals according to the availability of glycoside donors, then the nature of the polysaccharide should vary according to the concentration of glycoside donors and thus according to the nature of the carbon growth source. This was not so in the case of the extracellular heteropolysaccharide of *Aero. aerogenes* and therefore a mechanism of this type is unlikely to occur.

Rather, there appear to be two possible mechanisms:

(1) Synthesis carried out by a series of completely specific enzymes. The number of enzymes involved would depend on the complexity of the polysaccharide; even for the simplest type of regular structure (e.g. a linear chain with regularly spaced branching points) at least three specific enzymes would be required. Preliminary results of a structural investigation of the extracellular polysaccharide of *Aero. aerogenes* indicate a highly branched complex molecule, and therefore that a large number of enzymes would be required in order to synthesize this polysaccharide invariably.

(2) Synthesis carried out by a template mechanism as envisaged for protein synthesis. Such a template would probably involve deoxyribonucleic acid in view of the evidence of type transformation in the pneumococcus and other organisms (summarized by Austrian, 1952).

Since the ability to synthesize an extracellular polysaccharide and therefore form a mucoid colony is apparently controlled by a single mutable step, and thus according to current views by one enzyme, the former mode of synthesis involving a large number of enzymes is less likely. Thus the evidence favours the idea that heteropolysaccharide synthesis is carried out by some template mechanism, and is thus different from the mechanism of homopolysaccharide formation.

SUMMARY

1. The extracellular slime polysaccharide of *Aerobacter aerogenes* A 3 (S 1) (Klebsiella Type 54) was isolated and purified and shown to contain three main component sugars: D-glucose (50 %), L-fucose (10 %) and an unidentified uronic acid (29 %).

2. The bacterium was grown in presence of a variety of carbohydrates as the sole carbon and energy source and the polysaccharides produced from each were isolated. The composition was found to be invariable. The implications of this fact upon the nature of heteropolysaccharide synthesis is discussed.

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Studies on Fructosans. Part VI. The Degradation of Fructosans in Aqueous Solution.*

By G. O. Aspinall and R. G. J. Telfer.

[Reprint Order No. 5855.]

It has been shown that the degradation of fructosans in hot aqueous solution involves the formation of small quantities of acidic materials, which cause the fructosans to undergo slow hydrolysis. The oligosaccharides, formed from the partial breakdown of the fructosan from perennial rye-grass, have been examined. The isolation of sucrose provides further evidence that in the grass levans the chains of fructofuranose residues are terminated by a non-reducing glucopyranose unit.

It has been shown in Parts II, III, and IV of this series (Arni and Percival, *J.*, 1951, 1822; Laidlaw and Reid, *J.*, 1951, 1830; Aspinall, Hirst, Percival, and Telfer, *J.*, 1953, 337) that fructosans are degraded in aqueous solution at 100° and that after several hours the products consisted of fructose, glucose, and a series of oligosaccharides, amongst which sucrose was suspected. The present investigation was undertaken to study such degradations in greater detail and to obtain further information about the role of glucose in fructosan molecules. The isolation of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose from the hydrolysis of methylated inulin (Hirst, McGilvray, and Percival, *J.*, 1950, 1297) and of the methylated levan from cocksfoot grass (*Dactylis glomerata*) (Part IV, *loc. cit.*) showed that terminal non-reducing glucopyranose residues are present in these fructosans. Although, in the former case, evidence was also obtained for the presence of 2 : 4 : 6-tri-*O*-methyl-D-glucose in the hydrolysate of the methylated polysaccharide, it seems probable that this sugar arose from incomplete methylation as it has been shown by periodate oxidation (Aspinall and Telfer, *Chem. and Ind.*, 1953, 490) that no 1 : 3-linked glucose residues are present in inulin. Biogenetic evidence from the investigations of Bacon and Edelman (*Biochem. J.*, 1951, **48**, 114; **49**, 446, 529) and of Dedonder (*Compt. rend.*, 1950, **230**, 549, 997; 1951, **231**, 790; **232**, 1134, 1142) indicates that fructosans are built up in the plant from sucrose by enzymic transfructosidation, and thus provides further support for the view that terminal glucopyranose residues are present in fructosan molecules linked to the penultimate fructofuranose residues as in sucrose.

The degradation of two fructosans has been studied. Inulin was extracted from dahlia tubers with hot water and purified by deposition from aqueous solution on cooling. The levan from perennial rye-grass (*Lolium perenne*) was isolated from the sample of grass used by Laidlaw and Reid (*loc. cit.*) in their investigations. Both fructosans gave fructose and small quantities of glucose (2·8% and 2·0%, respectively) on hydrolysis. The degradation in aqueous solution at 100° was followed by observing changes in pH and optical rotation, and by periodic chromatographic examination of the products. The same general pattern of breakdown was observed with both fructosans; a gradual fall in pH was accompanied by a change in optical rotation with the formation at first of oligosaccharides and finally of fructose and glucose, complete breakdown occurring after about 24 hours. In addition, chromatographic evidence showed the presence of small quantities of difructose anhydrides. These substances were formed more readily from the hydrolysis

* A preliminary account of some of the following results has appeared elsewhere (*Chem. and Ind.*, 1952, 1244). Part V, *J.*, 1954, 2364.

of inulin. A parallel experiment showed that sucrose undergoes similar breakdown in aqueous solution at 100°, no sucrose being present after 30 hours. Typical data for the degradation of inulin are recorded in the Table.

The degradation of inulin in aqueous solution.

	Time (hr.)	pH	$[\alpha]_D$	Paper chromatography *					
				U	O	D + T	F	G	DFA
Inulin in 3% soln. at 100°	0	6·28	−40·3°	3	—	—	—	—	—
	4	5·14	−39·3	3	2	1	—	—	—
	8	4·40	−45·9	3	2	2	1	—	—
	12	3·93	−60·9	3	2	2	3	1	—
	16·5	3·70	−79·1	—	1	2	3	1	1
	25	3·35	−84·2	—	—	1	3	1	2
Inulin in 3% soln. at 100° in N_2	10	6·11	−44·6	3	1	—	—	—	—
	25	5·36	−47·7	3	2	1	1	—	—
	42	4·88	−53·6	3	2	2	2	—	—
	52	4·66	−67·1	2	2	2	3	1	1
	75	4·24	−78·5	—	1	1	3	1	2
Inulin in phosphate buffer (pH 6·80)	0	6·80	−38·5	3	—	—	—	—	—
	16	6·76	−39·2	3	—	—	—	—	—
	32	6·48	−40·0	3	—	—	—	—	—

* Numbers denote relative intensity, 3 denoting the greatest. U, unchanged fructosan; O, higher oligosaccharides; D + T, di- and tri-saccharides; F, fructose; G, glucose; DFA, difructose anhydrides.

The fall in pH together with chromatographic indication of the presence of acidic breakdown products showed that degradation of the fructosans was, at least in the later stages, an acid hydrolysis. When inulin was heated in phosphate buffer (pH 6·8) no oligosaccharides or monosaccharides were formed, although the dark coloration of the solution showed that some decomposition had occurred. It was also shown that the presence of atmospheric oxygen is an important factor in initiating the degradation; when inulin was heated in aqueous solution through which nitrogen was bubbled the breakdown occurred much more slowly, and after 60 hours considerable quantities of oligosaccharides were still present.

Preliminary chromatographic examination of the products of partial degradation of the levan from perennial rye-grass showed that an optimum yield of oligosaccharides, travelling on the chromatogram at the same rate as or slower than sucrose could be obtained if the reaction was stopped after 6 hours. The oligosaccharides, which, from their rate of movement on the chromatogram, appeared to be di-, tri-, and tetra-succharides, were separated chromatographically, eluted from the paper, and hydrolysed. The amounts of fructose and glucose thus obtained suggested that the " spots " on the chromatogram contained mixtures of oligosaccharides, each spot containing a sugar giving only fructose on hydrolysis, in addition to a sugar giving both fructose and glucose. Further investigation showed that each spot could be at least partially resolved into two components. The levan was heated at 100° in aqueous solution for 6 hours and the hydrolysis products were fractionated successively on charcoal–Celite (Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677) by elution with water and aqueous ethanol, and by partition chromatography on cellulose (Hough, Jones, and Wadman, *J.*, 1949, 2511). In this way several oligosaccharide-containing fractions were obtained. In some cases, these fractions required further separation by partition on filter sheets before the individual components were isolated.

From these extensive fractionations four sugars of particular interest were isolated: (i) a reducing disaccharide, which gave only fructose on both acid and enzymic hydrolysis; (ii) a reducing trisaccharide, which gave only fructose on acid hydrolysis, and fructose and the afore-mentioned disaccharide on incubation with yeast invertase; (iii) sucrose (identified by its physical constants and by conversion into the octa-acetate); and (iv) a non-reducing trisaccharide, which on hydrolysis gave fructose and glucose in the ratio of 2 : 1, and on partial hydrolysis gave fructose and sucrose together with traces of glucose and the reducing disaccharide. In view of their derivation from a fructosan of known

general structure, it is highly probable that the reducing di- and tri-saccharides contain D-fructofuranose residues linked through positions $C_{(2)}$ and $C_{(6)}$, and experiments to establish the mode of linkage are at present in progress. Again, it is probable that the non-reducing tris accharide is identical with the trisaccharide, kestose, synthesised from sucrose by yeast invertase and shown by Albon, Bell, Blanchard, Gross, and Rundell (*J.*, 1953, 24) to be *O*-α-D-glucopyranosyl-(1→2)-*O*-β-D-fructofuranosyl (6→2)-β-D-fructofuranoside. Evidence was also obtained for the presence in very small yield of a fructosylglucose. It is doubtful, however, if this sugar is of structural significance and it is likely that the sugar arose either as a reversion product or more probably by epimerisation from the reducing disaccharide during prolonged contact with the pyridine-containing chromatographic solvent. In a control experiment, fructose and glucose were heated together in aqueous solution but no evidence could be obtained for the formation of reversion products.

The isolation of sucrose from the partial hydrolysis of the levan from perennial ryegrass provides conclusive evidence that glucose residues are present in this polysaccharide as an integral part of the molecule. Schlubach and Holzer (*Annalen*, 1953, **578**, 207) claim to have isolated a fructosan from *Lolium perenne* devoid of glucose residues, and it is, indeed, possible that some fructosan molecules may contain no glucose. The present investigation, however, stresses the need for the utmost caution in handling these extremely labile polysaccharides lest inadvertent scission of the fructosan chain results in loss of the glucose-containing moiety. The evidence from both structural investigations and studies of enzymic transfructosidation shows that the majority, at least, of fructosan molecules, both of the inulin and of the levan type, contain terminal glucose residues linked as in sucrose.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the solvent systems: (*A*) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3; v/v; top layer); (*B*) butan-1-ol–ethanol–water (4 : 1 : 5; v/v; top layer); and (*C*) ethyl acetate–acetic acid–water (3 : 1 : 3; v/v; top layer). Sprays of aqueous aniline oxalate and naphtharesorcinol in hydrochloric acid were used to detect aldoses and ketoses, respectively.

Isolation of the Polysaccharides.—(a) *Inulin.* Dahlia tubers (variety "Crimson Flag") were extracted by Hirst, McGilvray, and Percival's method (*loc. cit.*). The inulin, which separated on cooling from aqueous solution, had $[\alpha]_D^{15}$ −40·3° (*c*, 2·4 in H_2O). Chromatographic examination of the hydrolysate (Hirst and Jones, *J.*, 1949, 1659; Duff and Eastwood, *Nature*, 1950, **165**, 848) in solvent *C* showed the presence of fructose (97·2%) and glucose (2·8%).

(b) *Levan.* The fructosan from *Lolium perenne* was isolated as described by Laidlaw and Reid (*loc. cit.*).

Degradation of Inulin in Aqueous Solution.—Inulin (1·5—2·0 g.) was dissolved in water (50 c.c.), and the solution heated on the water-bath and examined periodically for changes in optical rotation and pH, samples being withdrawn for chromatographic examination in solvent *A*. The results are given in the Table. In some cases, oligosaccharides, which appeared, from their rate of movement, to be di-, tri-, and tetra-saccharides, were eluted from the chromatogram and hydrolysed with aqueous 1% oxalic acid, and the hydrolysates examined chromatographically in solvent *C*. Fructose and glucose were present in each hydrolysate, but visual estimates of the ratio of the sugars suggested that each "discrete spot" on the chromatogram contained two oligosaccharides, one giving fructose and glucose and the other giving only fructose on hydrolysis. The inulin solution was also tested periodically with the ammonium thiocyanate–ferrous ammonium sulphate reagent (Young, Vogt, and Nieuland, *Ind. Eng. Chem. Anal.*, 1936, **8**, 198), but in no case were hydroperoxides present.

Inulin (1·5 g.) was heated in phosphate buffer solution (50 c.c.; pH 6·8) and the reaction was followed as described previously. Although the solution darkened considerably no breakdown products could be detected chromatographically. The degradation of inulin in unbuffered aqueous solution through which nitrogen was bubbled was similar to that in air but much slower (see Table).

Degradation of Levan in Aqueous Solution.—The degradation of *Lolium perenne* levan in aqueous solution was followed in the manner described for inulin, and the reaction pattern was essentially similar. In a typical experiment the following changes were observed: $[\alpha]_D^{16}$ −42·8° (initial value), −36·3° (4 hr.), −48·6° (8 hr.), −72·6° (16 hr.), −79·6° (24 hr., const.); pH 5·01 (initial value), 4·48 (4 hr.), 4·11 (8 hr.), 3·54 (16 hr.), 3·20 (24 hr.). Chromatographic

examination of the product in solvent *B*, followed by development with methyl-red–methylene-blue (Conway and Byrne, *Biochem. J.*, 1933, **27**, 419), showed the presence of two acids, one of which travelled at the same rate as lactic acid.

Degradation of Sucrose in Aqueous Solution.—A solution of sucrose (1·7 g.) in water (50 c.c.) was heated on the water-bath. The following changes were observed: $[\alpha]_D^{16}$ +65·3° (initial value) ⟶ −12·3° (36 hr., const.); pH 5·98 (initial value) ⟶ 3·32 (36 hr.). Chromatographic examination of the solution showed gradual breakdown with the formation of glucose and fructose, and after 30 hr. sucrose could no longer be detected. Two acids were also detected.

Large-scale Degradation of Levan and Separation of Oligosaccharides.—A solution of the levan (50 g.) in water (1·4 l.) was heated on the water-bath for 6·5 hr. {$[\alpha]_D$ −41·1° ⟶ −48·6° (6 hr.); pH 4·40 ⟶ 4·08 (6 hr.)}. The cooled solution was neutralised with barium carbonate, and the clear filtrate was concentrated; chromatographic examination of the syrup showed the presence of fructose and a series of oligosaccharides.

A solution of the syrup in water (500 c.c.) was poured on to charcoal–Celite (38·5 × 4·6 cm.) (Whistler and Durso, *loc. cit.*). The aqueous eluate, however, contained oligosaccharides in addition to monosaccharides and appropriate portions were, therefore, combined to give three fractions. Fraction *A* (10·2 g.) was shown chromatographically to contain only fructose and glucose and was not examined further. Fraction *B* (23·0 g.) contained fructose, glucose, sucrose, and a sugar having $R_{sucrose}$ 1·18. Fraction *C* (3·2 g.) contained fructose, sucrose, and sugars having $R_{sucrose}$ 1·18 and 0·71. Elution of the column with 50% aqueous ethanol gave fraction *D* (14·1 g.), which contained a sugar having $R_{sucrose}$ 0·71 and slower-moving oligosaccharides. Fraction *B* was further separated on charcoal–Celite (62 × 4·5 cm.), elution with water giving fraction *B*(i) (17·2 g.) containing fructose and glucose (trace), and elution with 5% aqueous ethanol giving fraction *B*(ii) (4·42 g.) containing a sugar having $R_{sucrose}$ 1·18 and *B*(iii) (0·546 g.) containing sucrose and sugars having $R_{sucrose}$ 1·18 and 0·85. Fraction *C* was separated on cellulose (70 × 3 cm.), solvent *A* being used, to give fractions containing fructose, glucose, and substances travelling on the chromatogram faster than fructose (probably difructose anhydrides), four oligosaccharide-containing fractions (1—4) and a fraction *C*(i) (0·914 g.) containing sucrose and the sugar of $R_{sucrose}$ 1·18. Fractions *B*(iii) and *C*(i) were combined and separated on cellulose (100 × 1·7 cm.) to give fractions containing glucose, fructose, and (probably) difructose anhydrides, and two further oligosaccharide-containing fractions (5 and 6). In some fractions traces of an unidentified substance were present; this travelled on the chromatogram considerably faster than fructose and gave a blue coloration with naphtharesorcinol and hydrochloric acid.

Examination of Oligosaccharide-containing Fractions.—Fraction 1. The syrup (0·442 g.), which reduced Fehling's solution and ammoniacal silver nitrate, had $[\alpha]_D^{17}$ −20·8° (*c*, 4·0 in H_2O) and $R_{sucrose}$ 1·18 in solvent *A*. Both mild acid hydrolysis and incubation with yeast invertase (B.D.H. " Invertase Concentrate ") gave only fructose. The rate of movement on the chromatogram indicated that the sugar was a disaccharide, and its derivation from a fructosan of known general structure suggests that the sugar was 6-*O*-β-D-fructofuranosyl-D-fructofuranose (referred to as fructobiose).

Fraction 2. Chromatographic examination of the syrup (0·242 g.) showed the presence of four sugars, probably (*a*) fructobiose, (*b*) sucrose, (*c*) fructosylglucose ($R_{sucrose}$ 0·85), (*d*) fructotriose. Separation of small quantities of the sugars was effected on the chromatogram by using solvent *A*, (*a*), (*c*), and (*d*), but not (*b*), reducing ammoniacal silver nitrate. On mild acid hydrolysis or incubation with yeast invertase (*a*) and (*d*) gave only fructose, while (*b*) and (*c*) gave glucose and fructose.

Fraction 3. The chromatographically pure syrup (0·393 g.) had $R_{sucrose}$ 0·71 in solvent *A*. The sugar had $[\alpha]_D^{16}$ −21·1° (*c*, 3·18 in H_2O) and reduced Fehling's solution and ammoniacal silver nitrate. Mild acid hydrolysis gave only fructose, and incubation with yeast invertase gave fructose and fructobiose. It seems probable that the sugar was 6-*O*-β-D-fructofuranosyl-6-*O*-β-D-fructofuranosyl-β-D-fructofuranose (referred to as fructotriose).

Fraction 4. Chromatographic examination showed the presence of two trisaccharides and separation on filter sheets with solvent *A* gave fractions (*e*) (450 mg.), having $R_{sucrose}$ 0·71 (identical with fructotriose), and (*f*) (40 mg.), having $R_{sucrose}$ 0·60. Fraction (*f*) and $[\alpha]_D^{18}$ +24° (*c*, 0·5 in H_2O) and was non-reducing towards Fehling's solution and ammoniacal silver nitrate. Incubation with yeast invertase gave glucose and fructose, and quantitative estimation (Hirst and Jones, *loc. cit.*) of the acid hydrolysate showed glucose and fructose to be present in the ratio 1 : 2. A portion of the syrup (10 mg.) was dissolved in water (5 c.c.), and the solution was heated at 100° with Amberlite resin IR-100 (0·5 g.). After 30 min. chromatographic

examination showed fructose and sucrose, together with traces of glucose and fructobiose. Hydrolysis to fructose and glucose was complete after 1 hr. It is probable that the sugar was *O*-α-D-glucopyranosyl-(1→2)-*O*-β-D-fructofuranosyl (6→2)-β-D-fructofuranoside (kestose) (Albon, Bell, Blanchard, Gross, and Rundell, *loc. cit.*). Albon *et al.* record $[\alpha]_D^{20}$ +27·3° for the crystalline sugar.

Fraction 5. The syrup (0·553 g.) contained sucrose and sugars having $R_{sucrose}$ 1·18 and 0·85. The sugars were separated on filter sheets, solvent *A* being used, to give fractions 5(*a*) (228 mg.), which travelled on the chromatogram at the same rate as fructobiose, 5(*b*) (287 mg.), which travelled on the chromatogram at the same rate as sucrose, and 5(*c*) (31 mg.). Fraction 5(*b*) crystallised from aqueous ethanol and had m. p. (and mixed m. p. with sucrose) 184—185° (Found : C, 42·2; H, 6·4. Calc. for $C_{12}H_{22}O_{11}$: C, 42·1; H, 6·4%). A sample was hydrolysed with aqueous 1% oxalic acid {$[\alpha]_D$ +59° ⟶ −24° (1 hr., const.)}, and quantitative estimation (Hirst and Jones, *loc. cit.*) of the hydrolysate showed glucose and fructose to be present in the ratio 1 : 1·05. The identity of the sugar was confirmed by an *X*-ray powder photograph (by courtesy of Dr. C. A. Beevers), which was identical with that of sucrose, and by conversion into sucrose octa-acetate, m. p. and mixed m. p. 72—73°, η_D^{20} (fused crystals) 1·4602, $[\alpha]_D^{17}$ +60° (*c*, 1·1 in $CHCl_3$) (Found : C, 49·5; H, 5·8; Ac, 50·3. Calc. for $C_{28}H_{38}O_{19}$: C, 49·5; H, 5·6; Ac, 50·7%).

Fraction 6. The syrup (31 mg.) was combined with fraction 5(*c*), both fractions containing a sugar having $R_{sucrose}$ 0·85 in solvent *A* together with traces of other sugars. The sugar reduced Fehling's solution and ammoniacal silver nitrate, and gave glucose and fructose on treatment with yeast invertase. Quantitative estimation of the acid hydrolysate showed glucose and fructose to be present in the ratio of 1 : 1·27. Reduction of alkaline hypoiodite (55% of theoretical for a disaccharide) showed that the major component of the syrup was a fructosylglucose.

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Gum Ghatti (Indian Gum). The Composition of the Gum and the Structure of Two Aldobiouronic Acids derived from it.

By G. O. Aspinall, E. L. Hirst, and A. Wickstrøm.

[Reprint Order No. 5856.]

Gum ghatti on hydrolysis yields a mixture of L-arabinose (5 mols.), D-galactose (3 mols.), D-mannose (1 mol.), xylose (½ mol.), D-glucuronic acid (1 mol.), and traces (below 1%) of methylpentose. On graded hydrolysis the gum gives two aldobiouronic acids, namely 6-*O*-β-D-glucuronosyl-D-galactose and 2-*O*-β-D-glucuronosyl-D-mannose. Oxidation of the gum with periodate has been studied.

The chemistry of gum ghatti (Indian gum) from *Anogeissus latifolia*, Wall (family, Combretaceæ), was investigated by Hanna and Shaw (*Proc. S. Dakota Acad. Sci.*, 1941, **21**, 78) who reported the presence of pentosan (50%) and galactose or galacturonic acid (12%) in the gum. They isolated L-arabinose after partial hydrolysis with acid and obtained a resistant aldobiouronic acid of equivlaent weight 352. In the present work a beginning has been made in the study of the detailed molecular structure of this important gum.

A purified sample of the gum had an equivalent weight of about 1600 (by titration with alkali) and on complete hydrolysis yielded L-arabinose, D-galactose, D-mannose, and D-glucuronic acid. Some xylose and a trace of methylpentose were also present but it is not certain whether these residues are present in the molecular structure.

The uronic anhydride content, determined by the carbon dioxide liberated by 19% hydrochloric acid at 145° (McCready, Swenson, and Maclay, *Ind. Eng. Chem. Anal.*, 1946, **18**, 290), was 12·0% (calculated for a substance of equivalent weight 1600, 11·0%). The pentosan content of the gum acid was 46·4%, calculated as anhydroarabinose from the liberated furfuraldehyde determined as thiobarbiturate (Marshall and Norris, *Biochem. J.*, 1937, **31**, 1293), account being taken of the furfuraldehyde yielded by the uronic acid residues (Norris and Resch, *Biochem. J.*, 1935, **29**, 1590).

After complete hydrolysis of the gum, determinations of the sugars separated on paper chromatograms were carried out by the periodate method (Hirst and Jones, *J.*, 1949, 1659). The following results were obtained: arabinose 41·1%, xylose 2·7%, galactose 26·7%, mannose 8·3%, the figures in each case being calculated for the anhydro-sugar residue.

A gum acid with equivalent weight 1600 consisting of 5 arabinose residues, 3 galactose residues, 1 mannose residue, and 0·5 xylose residue per unit of uronic anhydride, would contain the following percentages of anhydro-sugars: arabinose 41·2% (found, 41·1%), galactose 30·4% (found, 26·7%), mannose 10·1% (found, 8·3%), xylose 4·1% (found, 2·7%), uronic anhydride 11·0% (found, 12·0%).

When the gum acid was boiled in aqueous solution 4 moles of L-arabinose per equivalent were readily removed, probably because they are present in the furanose form and located in the outer parts of the molecule. The remaining arabinose residue was less easily removed by autohydrolysis, leaving a resistant degraded gum containing some 4% of arabinose residues possessing an equivalent weight of about 1000.

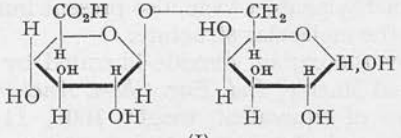
On reaction with periodate, 80% of the arabinose residues in the gum acid are oxidised; on the other hand, only about one third of the galactose residues are attacked. During the reaction *ca.* 3 moles of formic acid are produced per equivalent. These results are understandable if 80% of the arabinose residues in the gum molecule possess adjacent hydroxyl groups and are present as L-arabofuranose residues linked through $C_{(1)}$ and $C_{(5)}$. It is probable that the L-arabinosyl linkages are α in type, since on partial hydrolysis, the

specific rotation changes from $[\alpha]_D$ $-48°$ for the gum acid to $+6°$ for the degraded gum. It also follows that most of the galactose residues are engaged in linkages such as $C_{(1)}$–$C_{(3)}$ which exclude the presence of contiguous hydroxyl groups. In this connection it will be recalled that D-galactopyranose residues linked through $C_{(1)}$ and $C_{(3)}$ have been encountered in most of the plant gums previously examined. In view of the amount of formic produced during the oxidation with periodate it is likely that the D-galactose residue which is attacked is linked through positions $C_{(1)}$ and $C_{(6)}$.

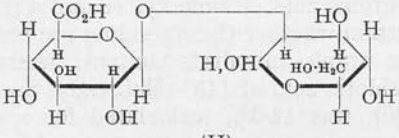
After partial hydrolysis of the gum acid with aqueous sulphuric acid a product containing two aldobiouronic acids was obtained. One of these contained D-mannose and a uronic acid whilst the other liberated D-galactose on further hydrolysis. Proof of their structure was obtained by methylation of the mixture of aldobiouronic acids and subsequent examination of the methylated sugars liberated on hydrolysis. The uronic acid fraction was then identified as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid after conversion into the crystalline methyl ester of 2 : 3 : 4-tri-*O*-methyl-D-saccharolactone. The neutral fractions were 3 : 4 : 6-tri-*O*-methyl-D-mannose and 2 : 3 : 4-tri-*O*-methyl-D-galactose, identified by their rates of movement on chromatograms and by their properties after isolation in the crystalline state.

The portion of the gum molecule most resistant to hydrolysis consists therefore of two aldobiouronic residues, namely 6-*O*-β-D-glucuronosyl-D-galactose (I) and 2-*O*-β-D-glucuronosyl-D-mannose (II), which appear to be present in approximately equal proportions, since the ratio galactose : mannose was 1 : 1 in the mixture of aldobiouronic acids obtained after partial hydrolysis of the gum with 0·16N-acid. The specific rotation of the methylated aldobiouronic acids indicates that the glucuronosyl junction is β in type.

In respect of the constituent sugars, gum ghatti resembles damson gum (Hirst and Jones, *J.*, 1938, 1174) and cherry gum (Jones, *J.*, 1939, 558), both of which are built up of residues of D-glucuronic acid, L-arabinose, D-xylose, D-galactose, and D-mannose. All three gums yield on partial hydrolysis the same aldobiouronic acid, 2-*O*-β-D-glucuron-



(I)



(II)

osyl-D-mannose (II). Gum ghatti and cherry gum show further similarity in the large proportion of L-arabinose residues present in their molecular structures, but much more work with both gums will be necessary before a detailed structural comparison will be possible. It is of interest in this connection that gum ghatti gives also on partial hydrolysis another aldobiouronic acid, namely 6-*O*-β-D-glucuronosyl-D-galactose (I) which is a characteristic feature of gum arabic and other gums of the *Acacia* group.

Experimental

Purification of the Gum.—The commercial sample of gum ghatti (obtained from Messrs. Eimer and Amend, New York) consisted of yellowish nodules, soluble in water with the exception of a few fragments of bark, etc. The aqueous solution gave a positive test for calcium. The gum acid was precipitated by pouring a filtered aqueous solution of the gum into ethanol, acidified with hydrochloric acid to about 0·1N. The precipitate was purified by the method described for damson gum (Hirst and Jones, *J.*, 1938, 1174) and was dried at 40—50° under reduced pressure.

It was noticed that during the precipitation a small portion of the gum acid (*ca.* 2%) separated as a finely dispersed suspension. This was separated, after addition of ether to the mixture, and showed the same analytical properties (equivalent weight, optical rotation, pentosan content) as the main batch, and on complete hydrolysis it liberated the same sugars. It appeared therefore to be identical with the main portion.

Analysis of the Purified Gum Acid.—The figures given below are mean values from a series of analyses carried out with two separately prepared batches; no significant difference in properties was observed between the two preparations: $[\alpha]_D^{20}$ $-50°$ (approx.) (as Na salt; *c*,

1·0 in H_2O); equivalent weight by acid acidimetry, 1600; carbon dioxide liberated when heated with 19% hydrochloric acid, 2·99% (corresponding to 12·0% uronic anhydride) (calc. for a polysaccharide of equiv. wt. 1600: CO_2, 2·75%, uronic anhydride, 11·0%); pentosan content, 46·4% (as anhydroarabinose; calc. from the amount of furfuraldehyde evolved under standard conditions); ash (as sulphate), <0·5%; N_2 (Kjeldahl), <0·4%; acetyl groups, <0·4%. The gum acid had an apparent methoxyl content (Zeisel estimation), 2·2%; but this was probably due to esterification during the precipitation by acid ethanol. The crude gum had a negligible OMe content (<0·1%). A small iodine number observed with the purified gum acid (5·6 ml. of 0·1N-iodine per 1·0 g.) may also be due to ethyl alcohol introduced during the purification. The gum acid did not reduce Fehling's solution.

Examination by Paper Chromatography of the Sugars produced on Complete Hydrolysis of the Gum Acid.—A solution (0·5%) of gum acid in 2N-sulphuric acid was heated for 24 hr. in a sealed tube immersed in a boiling-water bath. The solution was neutralised (Amberlite IR-4B) and then concentrated under reduced pressure. On chromatograms including reference substances and run with several solvent systems, the four sugars, galactose, mannose, arabinose, and xylose, were detected. Spots corresponding to more resistant parts of the gum (*e.g.*, oligosaccharides) were not observed. Another solution, obtained as described above, was neutralised by barium carbonate, followed by removal of the barium neutral filtrate by Amberlite IR-120. The solution was then concentrated under reduced pressure and examined on paper chromatograms obtained under the conditions described for the detection of uronic acids (Partridge, *Biochem. J.*, 1948, **42**, 238). Spots corresponding to glucuronic acid and glucurone were observed.

Quantitative Determination of the Sugars present in the completely Hydrolysed Solution.—After paper-chromatographic separation of the sugars, their quantities were determined by the periodate method (Hirst and Jones, *J.*, 1949, 1659), D-ribose being used as reference sugar. The figures are mean values from a series of chromatograms and indicate the proportions (calculated as anhydro-sugars) obtained from 100 parts of intact gum acid; galactose, 26·7%, mannose, 8·3%, arabinose, 34·0%, xylose, 2·7%.

These figures plus 11·0% uronic anhydride fall short of 100% and it was evident that decomposition had taken place during the hydrolysis. This is mostly due to loss of pentose since the figure for anhydroarabinose by the furfuraldehyde method was 46·4%. Subsequent experiments showed that most of the pentose is liberated during 60 min. in the sealed-tube experiments. It follows that the free arabinose was submitted to more than 20 hours of heating in a boiling-water bath in an acid medium. As shown in the following section it is, however, possible by using milder conditions of hydrolysis to obtain quantitative results for arabinose which are in good agreement with the furfuraldehyde determination.

Quantitative Determination of Arabinose after Autohydrolysis of the Gum Acid and after Hydrolysis of the Gum with 1·0N-*Formic Acid.*—A 2% aqueous solution of gum acid was heated in a boiling-water bath until the optical rotation became constant at $[\alpha]_D^{20}$ +49° after 44 hr. Paper chromatography showed that, in addition to the liberated arabinose and traces of xylose, galactose began to split off after 44 hr. Determination of the arabinose on the chromatograms showed that an amount corresponding to 34·4% of anhydroarabinose, or 4 residues per equivalent weight (1600), was liberated.

Hydrolysis of the gum acid with N-formic acid in a boiling-water bath for 20 hr. proceeded with little apparent decomposition. The hydrolysis products were separated chromatographically. As in the case of the autohydrolysis, xylose and galactose were liberated in amounts too small for accurate determinations. (*A*) Gum acid (544·6 mg.) and D-ribose (199·5 mg.) were dissolved in N-formic acid (10 ml.) and refluxed in a boiling-water bath for 20 hr. Arabinose liberated: 42·7% (calc. as anhydroarabinose). This amount did not increase during continued heating in 3N-formic acid. (*B*) D-Ribose (175·8 mg.) was added after the hydrolysis (20 hr.) of gum acid (573·0 mg.) in N-formic acid (10 ml.). Arabinose liberated: 39·5% (calc. as anhydroarabinose). It is likely that method (*A*) will give results slightly above the correct figure, whereas method (*B*) probably involves a slight error in the opposite direction. The mean value (41·1%; corresponding to 5 residues of arabinose per equiv.) is in reasonable agreement with the results of the furfuraldehyde determination (46·4%) when account is taken of the presence of xylose (*ca.* 3%) and rhamnose (*ca.* 1%) residues.

Examination of the Degraded Gum.—An aqueous solution of the gum acid was heated for 48 hr. in a boiling-water bath ($[\alpha]_D^{20}$ +49°). It was then filtered and concentrated to a thin syrup at 40—50° under reduced pressure. The syrup was poured into ethanol, and the precipitated polysaccharide washed repeatedly with hot methanol and dried at 40° under reduced

pressure (yield 25%). It had $[\alpha]_D^{20}$ +6° (as sodium salt; *c*, 1·0 in H_2O); equiv. (by acidimetry), 985; equiv. (by determination of uronic anhydride), 977. Pentosan, calculated as anhydro-arabinose, 3·7%, from furfuraldehyde determinations.

Determinations were made after paper-chromatographic separation of the sugars liberated on complete hydrolysis of the degraded gum (by heating it with 2N-sulphuric acid for 24 hr. in a sealed tube immersed in a boiling-water bath). The following mean values were obtained (calc. as percentage of anhydro-sugar in the intact degraded gum): galactose, 48·5%; mannose, 11%; arabinose, 3·5%; xylose, 1%. Account being taken of the presence of 17·2% of uronic anhydride, the total is less than 100%, owing probably to decomposition during hydrolysis. Approximately one sugar residue per equivalent is missing.

Isolation of L-*Arabinose and* D-*Galactose, by Stepwise Hydrolysis of the Gum Acid.*—L-*Arabinose.* After autohydrolysis and precipitation of the degraded gum acid, the mother liquor was concentrated under reduced pressure to a thick syrup, which was repeatedly extracted with boiling ethanol. The combined alcoholic extracts on concentration yielded crystalline L-arabinose (yield 10%), still contaminated with traces of xylose, but after recrystallisation from ethanol the substance was chromatographically homogeneous and then had m. p. and mixed m. p. 156—158°, $[\alpha]_D^{20}$ +103° (*c*, 1·0 in H_2O).

D-*Galactose.* Degraded gum acid was partly hydrolysed with sulphuric acid (2N) in a boiling-water bath. After 4 hr. the solution was neutralised with barium carbonate, filtered, concentrated (reduced pressure), and poured into ethanol. The barium salts were filtered off and the mother liquid was concentrated (reduced pressure) to dryness. On trituration of the residue with hot methanol crystalline D-galactose separated (yield 2%); chromatographically pure it had m. p. and mixed m. p. 162—165°. Further evidence that this was the D-isomer was provided by the isolation of *O*-methyl-D-galactoses on hydrolysis of the methylated degraded gum (unpublished results).

Oxidation of the Gum Acid by Periodate.—Portions (10 ml.) of an aqueous solution (0·0025N; neutral to methyl red) of the sodium salt of the gum acid were allowed to react with sodium metaperiodate (0·05M; 10 ml.) at room temperature in the dark. The periodate uptake was determined by the arsenite method, and the formic acid by titration with aqueous sodium hydroxide (methyl red as indicator) after addition of ethylene glycol. The following results were obtained as mean values of two series of analysis:

Time (hr.)	2	4	8	20	48	60	96
Periodate reduced (moles)	7·0	8·4	9·1	9·9	10·5	10·7	10·8
Univalent acid liberated (moles)/equiv. of gum acid	2·5	2·8	3·0	3·1	3·3	3·4	3·4

Examination of the Material remaining after Oxidation of the Gum with Periodate.—Preliminary qualitative determinations showed that some galactose, mannose, and arabinose still remained intact in the oxidised gum. But neither uronic acid nor xylose could be detected.

Gum acid (270 mg.) was oxidised with sodium metaperiodate (700 mg.) for 48 hr. The oxidation was stopped by the addition of ethylene glycol, and the solution was then dialysed against running water until inorganic ions had been removed (5 days). The solution was concentrated (reduced pressure) to about 30 ml., made acid (2N) with sulphuric acid, and heated for 20 hr. at 98°. It was neutralised with barium carbonate, and the concentrated filtrate was examined on paper chromatograms. Analyses showed that the following sugars were present: arabinose, 9·2%; galactose, 20·8%; mannose, 7·1% (the figures give the amount of each sugar remaining unoxidised expressed as percentages of the weight of the original gum acid).

Examination of the Product obtained by Graded Hydrolysis of the Gum.—Hydrolysis of the gum with aqueous sulphuric acid could not be followed beyond the early stages polarimetrically or by determination of the iodine number, since even with 0·16N-acid considerable destruction took place (brown colour and precipitate). After 24 hr. at 100°, the solution was neutralised and the acidic materials were isolated as the barium salts [Yields: (*a*) Product *A* from hydrolysis with 0·16N-acid; 15% with Ba content, 18·6%. (*b*) Product *B* from hydrolysis with 0·5N-acid; 8% with Ba content, 23·0%. (*c*) Product *C* from 0·9N-acid; 1·5%]. All three products liberated glucuronic acid, galactose, and mannose on further hydrolysis. Quantitative paper-chromatography showed that galactose and mannose were liberated in equimolecular proportions from product *A* and in the ratio 1 : 1·5 from product *B*. The barium content is too high to account for the presence of a trisaccharide (aldotriouronate) or a mixture of an aldobiouronic acid and a resistant oligosaccharide (barium calc. for aldobiouronate, 16·2%; for aldotriouronate, 11·7%).

Barium ions were removed from an aqueous solution, with Amberlite IR-120, and the

resulting solution was examined on paper-chromatograms run with the solvent systems (I) (amyl alcohol–pyridine–water; 7 : 7 : 6), and (II) (ethyl acetate–formic acid–acetic acid–water; 18 : 1 : 3 : 4) (both systems, " one-phase "). Two spots showing reducing and acid properties (bromophenol blue) were observed, moving at rates characteristic of disaccharides; in solvent (I) $R_{galactose}$, 0·45 and 0·55 (galactose, 1·00); in solvent (II) $R_{glucurone}$, 0·14 and 0·23 (glucurone 1·00). Glucuronic acid which is present in traces was clearly separated from the other spots in system (II).

Chromatograms with larger quantities of product *A* were run [solvent (I)] on thick paper (Whatman 3MM). The two spots were eluted and on hydrolysis of the eluates the upper spot yielded glucoronic acid and galactose whereas the lower spot yielded glucuronic acid and mannose (paper-chromatography). Final proof of the presence of two distinct aldobiouronic acids in the mixture emerged from the methylation studies described below.

Large-scale Preparation of the Barium Aldobiouronates.—Gum acid (100 g.) was dissolved in 0·45N-sulphuric acid (2 l.) and heated in a boiling-water bath for 24 hr. The filtered solution was neutralised with barium carbonate (150 g.) at 80° with stirring, and the liquid portion was decanted, centrifuged, and concentrated at 40—50° (reduced pressure) to a syrup which was poured into alcohol. The precipitated barium salts were washed with hot alcohol (crude yield, 25 g.), purified by 3 successive precipitations and washed with alcohol (yield, 10 g.) [Found: OMe, negligible; adsorbed free sugars (by paper-chromatography)—traces of galactose and arabinose, and traces of glucuronic acid (all below 1%); Ba, 23·0% (calc. for barium aldobiouronate, 16·2%); uronic anhydride, 44·0% (calc. for barium aldobiouronate, 41·5%); galactose (as anhydro-sugar), 15·8%; mannose (as anhydro-sugar), 23·7%; (both determined by paper-chromatography after hydrolysis)]. The total hexose anhydride is therefore 39·5% (calc. for a barium aldobiouronate, 38·2%).

Isolation of a Methylpentose as Hydrolysis Product.—The alcoholic washings from the large-scale preparation of the barium salts of aldobiouronic acids (see previous section) were concentrated to dryness under reduced pressure. The product gave on chromatographic examination, in addition to the spots expected, a weak spot which moved at the rate of rhamnose. Determination by Nicolet and Shinn's method (*J. Amer. Chem. Soc.*, 1941, **63**, 1456) showed the presence of methylpentose in amount corresponding to about 1% of the gum.

Methylation of the Aldobiouronic Acids.—The barium salts (9 g.) were dissolved in water (100 ml.) and methylated three times with dimethyl sulphate and sodium hydroxide in an atmosphere of nitrogen with vigorous stirring. (1) Dimethyl sulphate (100 ml.) was added and then sodium hydroxide (200 ml.; 30%) dropwise at a temperature below 30°. (2) Solid sodium hydroxide (50 g.) was dissolved in the mixture the following day and dimethyl sulphate (100 ml.) was added dropwise at a temperature below 35—40°. The mixture was heated for 30 min. on a boiling-water bath, cooled, partly neutralised (H_2SO_4), and filtered. (3) The concentrated (at reduced pressure) filtrate was again methylated by dimethyl sulphate (50 ml.) and sodium hydroxide (100 ml.; 30%) at below 40°. The mixture was finally heated to 80° for 30 min., cooled, acidified with sulphuric acid, and repeatedly extracted with chloroform. The combined extracts were concentrated to a syrup under reduced pressure, and the residue (5 g.) was further methylated with methyl iodide and silver oxide. The product (a yellow, viscid syrup; 4·7 g.) was distilled, giving the following fractions (temperatures are those of the heating bath): (I) a bright yellow, mobile syrup (0·80 g.), b. p. 185—190°/0·1 mm. (Found: OMe, 49·8%); (II) nearly solid, brownish yellow (1·4 g.), b. p. 200°/0·03 mm. (Found: OMe, 49·9%), $[\alpha]_D^{20}$ −24·2° (*c*, 5 in MeOH); (III) residue, b. p. >230°/0·03 mm. (Found: OMe, 50·1%).

Samples of the fractions were hydrolysed and the methylated sugars liberated were examined on paper-chromatograms. None of the fractions gave tetramethylgalactose or tetramethylmannose. Fraction II gave spots of methylated uronic acid, trimethylgalactose, R_G 0·65, and a trimethylmannose, R_G 0·80. Fraction I gave mainly methylated uronic acid and traces of the two trimethyl sugars. The residue (fraction III) gave the same spots as fraction II, but in addition there were two unidentified sugars (traces only), having R_G 0·50 and 0·78, respectively.

Separation and Identification of the Hydrolysis Products of the Methylated Aldobiouronic Acids (Fraction II).—Fraction II (1·2 g.) was hydrolysed with N-sulphuric acid (25 ml.) for 13 hr. in a boiling-water bath. The solution was neutralised ($BaCO_3$), filtered, and evaporated, leaving a yellowish powder (1·0 g.), which was exhaustively extracted with dry ether. The product was a chromatographically-homogeneous barium salt of methylated glucuronic acid (*Y*) (0·50 g.).

The ethereal extract on evaporation gave a yellowish syrup (*X*) (0·50 g.), which was separated

into its constituents on cellulose (working dimensions, 2·5 × 75 cm.), *n*-butanol–light petroleum (b. p. 100—120°) (30/70 by vol.), saturated with water, being used as mobile phase. The eluate was collected in portions (6—7 ml.), and two fractions were obtained: (i) (0·10 g.), 3 : 4 : 6-tri-*O*-methyl-D-mannose, R_G 0·80, m. p. and mixed m. p. 101—102°, $[\alpha]_D^{20}$ +10° (*c*, 2 in H_2O); (ii) (0·05 g.), 2 : 3 : 4-tri-*O*-methyl-D-galactose, R_G 0·64 (Hirst and Jones, *Discuss. Faraday Soc.*, 1949, **7**, 271). Fraction (ii) crystallised as the hydrate when kept. After recrystallisation from ether, it had m. p. 78°, with some sintering at 73° (see Smith, *J.*, 1939, 1734) and showed a large depression of m. p. when mixed with 2 : 4 : 6-tri-*O*-methyl-D-galactose (R_G 0·67).

The barium salt (*Y*) was dissolved in water, and barium ions were removed with Amberlite IR-120. The tri-*O*-methyl-D-glucuronic acid remaining in solution was oxidised with bromine water to 2 : 3 : 4-tri-*O*-methyl-D-saccharic acid (0·25 g.) which was esterified with methanolic hydrogen chloride. The ester was distilled (b. p. 160°/0·3 mm.); the distillate ($[\alpha]_D$ positive) crystallised when nucleated with the methyl ester of 2 : 3 : 4-tri-*O*-methyl-D-saccharolactone, m. p. and mixed m. p. 109—110°. The two aldobiouronic acids obtained by partial hydrolysis of the gum are therefore 2-*O*-β-D-glucuronosyl-D-mannose and 6-*O*-β-D-glucuronosyl-D-galactose.

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The Constitution of a Modified Starch from Malted Barley.

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The starch from malted barley has been isolated and shown to contain 26·0% of amylose. Methylation showed that the amylose consisted of unbranched chains containing an average of *ca.* 330 glucose residues. Methylation end-group assay, supported by β-amylolysis and periodate oxidation, indicated that the amylopectin contained the normal type of branched structure, but with one non-reducing terminal group per 18 ± 1 glucose residues. It is concluded that during the malting of barley the amylopectin has undergone enzymic attack causing shortening of the outer chains but with retention of the branched structure, while the amylose component has been relatively little degraded.

Structural investigations have recently been carried out on starches from a variety of sources. In this paper we report an examination of the starch from malted barley (a mixture of Plumage Archer and Spratt Archer varieties), carried out to determine the changes in structure of the starch during the malting process. The isolation was achieved without the use of reagents likely to cause degradation. The starch had an amylose content of 26·0%, a value significantly higher than that of the starch from the corresponding sample of barley and of the barley starch examined by McWilliam and Percival (*J.*, 1951, 2259). The most satisfactory separation of malted-barley amylopectin was achieved by using 20% aqueous pyridine saturated with butanol as the amylose precipitant (Higginbotham and Morrison, *Shirley Inst. Mem.*, 1948, **22**, 148). The amylopectin so obtained had an amylose content of not more than 0·5%. The amylose was isolated by fractionation of the starch with 15% aqueous pyridine (Whistler and Hilbert, *J. Amer. Chem. Soc.*, 1945, **67**, 1161), followed by successive reprecipitations as the butanol complex (Higginbotham and Morrison, *loc. cit.*).

Methylated amylopectin was prepared by standard methods and quantitative paper chromatography (Hirst, Hough, and Jones, *J.*, 1949, 928) of a small-scale hydrolysate showed the presence of tetra- (5·5%), tri- (86·1%), and di-*O*-methylglucose (8·4%). The mixture of methylated sugars obtained on hydrolysis of the methylated polysaccharide on a larger scale was fractionated by partition chromatography on cellulose, to give 2 : 3 : 4 : 6-tetra- (5·7%), 2 : 3 : 6-tri- (87·4%), and 2 : 3-di-*O*-methyl-D-glucose (4·3%), isolated as crystalline substances or as crystalline derivatives. In addition there was obtained a small fraction (2·6%) containing a mixture of 2 : 6- and 3 : 6-di-*O*-methylglucose. The quantity of tetra-*O*-methylglucose corresponded to the presence of one non-reducing terminal group per 18—19 glucose residues. It is doubtful whether the mixture of 2 : 6- and 3 : 6-di-*O*-methylglucose is of structural significance, since they may have arisen from incomplete methylation of the polysaccharide and by demethylation during hydrolysis. The quantity of 2 : 3-di-*O*-methyl-D-glucose isolated indicates that the majority, at least, of the interchain linkages were through position 6. The yield of formic acid from periodate oxidation of the amylopectin corresponded to one non-reducing group per 18 ± 1 glucose residues, a value in good agreement with that from the methylation data. Estimation of the glucose produced on hydrolysis of the periodate-oxidised polysaccharide indicated that 0·63% of the glucose residues in the amylopectin were unattacked by periodate. It follows that at least 88% of the linkages between unit chains were 1 : 6-linkages.

Further evidence concerning the structure of the amylopectin was obtained from its degradation by β-amylase, when in two experiments 44% and 47% of the molecule was converted into maltose. This value for the β-amylolysis of malt amylopectin is significantly

lower than the values (52—62%) normally found for amylopectins (see Manners, *Quart. Rev.*, 1955, **9**, 73). As β-amylase only attacks the exterior chains of amylopectins, the enzymic degradation stopping at one or two glucose units from the inter-chain linkage, it can be calculated that the β-limit dextrin had an average chain length of 9—10. A value of 8—9 residues for the chain length of the limit dextrin was obtained on periodate oxidation of the polysaccharide isolated after β-amylolysis. This is in close agreement with the values previously found for amylopectin β-limit dextrins (cf. Halsall, Hirst, Hough, and Jones, *J.*, 1949, 3200).

The malted-barley amylose was methylated, initially with diazomethane and subsequently with the usual reagents, and hydrolysis of the methylated polysaccharide gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose (0·32%), 2 : 3 : 6-tri-*O*-methyl-D-glucose (98·0%), and a mixture of di-*O*-methylglucoses (1·6%). It is doubtful if the dimethyl sugars are of structural significance. The quantity of tetra-*O*-methylglucose corresponded to the presence of one non-reducing terminal group per 310 ± 20 glucose residues. A value of 330—345 for the average chain length of the amylose obtained from periodate oxidation experiments was in reasonable agreement with that from the methylation data. The malt amylose, therefore, consisted of an unbranched chain of *ca.* 330 glucose residues. It is interesting that this amylose is completely converted into maltose by β-amylase (the experiment was kindly carried out by Dr. D. J. Manners), and, therefore, does not contain the barrier to complete β-amylolysis found in some amyloses (Peat, Thomas, and Whelan, *J.*, 1952, 722).

Investigations were also carried out on the starch from the same sample of barley which had been used to prepare the malt. This barley starch had an amylose content of 22·6% and the amylopectin component had an average chain length of 26 glucose residues. β-Amylolysis of the amylopectin gave a 59% conversion into maltose, from which an average chain length of 10 can be calculated for the limit dextrin. A value of 9—10 glucose residues was obtained on periodate oxidation of the polysaccharide isolated after β-amylolysis of the barley amylopectin.

From these investigations the main structural features of the starch from malted barley emerge. The amylopectin has the type of branched structure that is now well established for this starch component. The average chain length (18 ± 1), however, is significantly shorter than that normally found for amylopectins and, in particular, is shorter than that found for the corresponding barley amylopectin (26 ± 1), a value in agreement with that previously determined by McWilliam and Percival (*loc. cit.*). It is apparent, therefore, that the exterior chains of the amylopectin have been partially removed by enzymic action during the malting of the barley. The chromatographic identification of glucose and maltose in the aqueous-ethanolic extract of the malted barley suggests that the action has been that of β-amylase in conjunction with maltase. On the other hand the isolation of a methylated amylopectin of high molecular weight indicates that there has been no appreciable α-amylolytic action causing scission of the interior chains during malting. An alternative interpretation that the amylopectin component is the product of complete breakdown and resynthesis is less likely in view of the similarity between the β-limit dextrins of the barley and the malted-barley starch. The apparent increase in the amylose content of the malt starch is fully accounted for by the loss of *ca.* 25% of the amylopectin component by shortening of the exterior chains, and it appears that the amylose has undergone relatively little degradation during malting, the polysaccharide isolated after fractionation being of a similar size to the barley amylose examined by McWilliam and Percival (*loc. cit.*). It is to be remembered, however, that the amylose examined may not be representative of all the amylose present in the malt starch, since only 55% of the total amount of this component present in the malt was isolated. Nevertheless any shorter-chain amyloses, which escaped precipitation during the fractionation, were of large enough molecular size to be estimated as amylose in the potentiometric iodine titration.

The general pattern of changes undergone by the starch in the malting process has now been established, but many problems remain unsolved. For instance, the present results do not show whether the degradation of the outer chains of the amylopectin has been completely random, or whether in parts of the macromolecule enzymic action has been

carried to one or two glucose units from the inter-chain linkage (as in β-amylolysis) while in other parts of the molecule no degradation has occurred. Although relatively little breakdown of the amylose component has so far been detected, a detailed comparison of the linear fractions from barley and from malted barley would be of value, since considerable changes in this component could occur before the achroic limit was reached.

Experimental

The following solvents were used to separate the sugars and their derivatives: (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3; top layer), (B) butan-1-ol–ethanol–water (4 : 1 : 5; top layer), and (C) benzene–ethanol–water (169 : 47 : 15; top layer). Blue values were determined by Hassid and McCready's method (*J. Amer. Chem. Soc.*, 1943, **65**, 1154) as modified by Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924). Potentiometric determinations of amylose were kindly carried out by Mr. D. M. W. Anderson and Dr. C. T. Greenwood (see Anderson and Greenwood, *J.*, in the press).

Isolation of Starch from Malted Barley.—Malted barley (mixture of Plumage Archer and Spratt Archer varieties; 1·5 kg.) was lightly pounded to split open the husk, most of the husk was blown off with a jet of air, and the starch granules were separated from further fibrous material by passage through a suitable sieve. The starchy material was extracted for 3 hr. with boiling aqueous ethanol to inactivate enzymes and to remove soluble sugars. The ethanolic extract was concentrated, and chromatographic examination of the resulting syrup showed the presence of glucose, fructose, arabinose, and maltose. The inactivated malt was dried, and ground in a " Raymond " laboratory mill, and a suspension in ethanol was passed through a 40-mesh sieve to remove the residual fibre. The separated starch granules were stirred with cold water for 3 hr. to remove water-soluble carbohydrates, the aqueous extract was decanted, and the crude starch was dried with ethanol and ether. After exhaustive extraction with boiling 1 : 19 aqueous methanol to remove fats, crude starch (400 g.) was isolated. Examination under the microscope showed the starch granules to be ovoid to spherical in shape, and many of them appeared to be slightly ruptured.

Purification of Starch.—(a) *Treatment with butanol and toluene.* Crude starch (120 g.) was stirred overnight in 0·1% aqueous sodium hydrogen sulphite (5 l.). Removal of the solid at the centrifuge gave a lower layer containing the heavier starch granules, and a pale brown protein-rich upper layer. The upper layer was removed as completely as possible, the starch layer was subjected to the same treatment three more times, and the starch was freeze-dried. As the starch still contained protein (2·5%), a sample (4 g.) was stirred in water (200 c.c.), and the suspension was shaken with butanol before being allowed to settle. The brown precipitate, obtained at the butanol–water interface was removed, and the procedure was repeated once with butanol and three times with toluene. Starch A (2·3 g.) was isolated by freeze-drying (Found: N, 0·05%).

(b) *Extraction with chloral hydrate.* Crude starch (20 g.) was extracted by the method of Meyer and Bernfeld (*Helv. Chim. Acta*, 1940, **23**, 875) to give starch B (14·3 g.) (Found: N, 0·03%). The starch had a blue value of 0·368.

Examination of Starches.—Starch A had $[\alpha]_D^{18}$ +154° (*c*, 0·9 in N-NaOH) and $[\alpha]_D^{18}$ +178° (*c*, 0·7 in 30% $HClO_4$). Potentiometric titration showed the presence of 26·0% of amylose. Periodate oxidation of a sample of the starch as described by Brown, Halsall, Hirst, and Jones (*J.*, 1948, 27) gave formic acid corresponding to one terminal non-reducing group per 25 glucose residues. The value corresponded to a chain length of 19 glucose residues in the amylopectin fraction.

Starch B had $[\alpha]_D^{16}$ +159° (*c*, 1·1 in N-NaOH) and $[\alpha]_D^{16}$ +182° (*c*, 0·78 in 30% $HClO_4$). Hydrolysis of a sample gave only glucose (97·6%). Potentiometric titration showed the presence of 27·8% of amylose.

Fractionation of Starch A.—Starch A (12 g.) was fractionated by Higginbotham and Morrison's method (*Shirley Inst. Mem.*, 1948, **22**, 148) with 4 : 1 aqueous pyridine and butanol. The amylose complex was separated, washed with water saturated with butanol, dispersed in water, and freeze-dried, to give an amylose-rich fraction (3·8 g.). The amylopectin was precipitated by pouring the supernatant liquor from the fractionation into ethanol (2 vols.), and the precipitate was dispersed in water and freeze-dried to give amylopectin A (5·4 g.). The amylose-rich fraction was dispersed in water, refractionated seven times with butanol (Higginbotham and Morrison, *loc. cit.*), and isolated as before, to give amylose A (1·1 g.).

Examination of Starch Fractions.—Amylopectin A. This fraction had $[\alpha]_D^{18}$ +147° (*c*, 0·5 in N-NaOH) and $[\alpha]_D^{18}$ +166° (*c*, 0·5 in 30% $HClO_4$) (Found: N, 0·05%). Potentiometric

titration showed the presence of 3·9% of amylose. Periodate oxidation of a sample gave formic acid corresponding to one terminal non-reducing group per 18 glucose residues.

Amylose A. This fraction had $[\alpha]_D^{18}$ +148° (*c*, 0·5 in N-NaOH) and $[\alpha]_D^{18}$ +198° (*c*, 0·7 in 30% $HClO_4$) (Found : N, 0·02%). Potentiometric titration showed that the amylose had a maximum iodine-binding power of 18·3%.

Large-scale Fractionation of Malted Barley Starch.—Preliminary fractionation of samples of the crude malted-barley starch showed that an amylose fraction with the highest blue value was obtained by fractionation of the starch with 85 : 15 water–pyridine (Whistler and Hilbert, *J. Amer. Chem. Soc.*, 1945, **67**, 1161), and that an amylopectin fraction of low amylose content was obtained by fractionation using 4 : 1 aqueous pyridine saturated with butanol (Higginbotham and Morrison, *loc. cit.*).

Crude malted-barley starch (30 g.) was suspended in cold water (200 c.c.), and the suspension was added slowly with vigorous stirring to water (2 l.) and pyridine (450 c.c.) at 90°. The solution was maintained at this temperature for 2·5 hr. to ensure maximum dispersion of starch, cooled to 70°, centrifuged at 2000 r.p.m. to remove insoluble fibrous material, and heated again to 90°. The solution was transferred to a Dewar flask and allowed to cool slowly for 60 hr., during which the amylose–pyridine complex separated. The products from four fractionations were combined and refractionated with water saturated with butanol (twice at an amylose concentration of 0·5%, six times at 0·2%, and four times at 0·1% concentration). The final amylose–butanol complex was dispersed in water (150 c.c.) and freeze-dried, to give amylose B (11·1 g.; this represents *ca.* 55% of the amylose originally present in the crude starch).

Crude malted-barley starch (60 g.) was suspended in water (200 c.c.), and the suspension was slowly added with vigorous stirring to water (1800 c.c.) and pyridine (400 c.c.) at 90°. The paste was stirred at this temperature for 3 hr., subjected to 5 minutes' high-speed stirring in an "Ato-Mix" disperser, cooled to 70°, and centrifuged to remove fibrous material. The paste was heated again to 90°, butanol (*ca.* 400 c.c.) was added slowly to saturate the solution, and the solution was transferred to a Dewar flask and allowed to cool slowly for 60 hr. The amylose complex was separated at the centrifuge and the supernatant liquid was concentrated under reduced pressure to 1500 c.c. and poured into ethanol (4500 c.c.). The precipitated polysaccharide was washed several times with water saturated with butanol, dispersed in water, and freeze-dried. Two such fractionations gave amylopectin B (41 g.).

Examination of malted-barley amylopectin.

Amylopectin B had $[\alpha]_D^{16}$ +149° (*c*, 1·1 in N-NaOH) and $[\alpha]_D^{16}$ +170° (*c*, 0·72 in 30% $HClO_4$). Hydrolysis of a sample gave only glucose (96·0%). The amylopectin had a blue value of 0·085, and potentiometric titration showed the presence of 0·5% of amylose (average of two determinations).

Methylation of the Amylopectin.—Amylopectin (24 g.) was methylated twelve times with methyl sulphate and sodium hydroxide solution under nitrogen at room temperature, and the product (24·4 g.; OMe, 43·0%) was fractionated in boiling chloroform–light petroleum (b. p. 60—80°) mixtures, to give a main fraction {20·7 g.; $[\alpha]_D^{15}$ +200° (*c*, 0·5 in $CHCl_3$); OMe, 43·2%}. Part of this fraction (6·0 g.) was methylated twice more with methyl iodide and silver oxide, to give methylated amylopectin {5·2 g.; $[\alpha]_D^{15}$ +200° (*c*, 0·5 in $CHCl_3$); OMe, 43·5%}, used in subsequent experiments. The methylated amylopectin had $\eta_{sp.}/c$, 2·21 (*c*, 0·4 in *m*-cresol), corresponding to an apparent *M* 320,000 (see Hirst and Young, *J.*, 1939, 1475).

Hydrolysis of Methylated Amylopectin and Separation of Methylated Sugars.—A sample of the methylated amylopectin (50 mg.) was hydrolysed successively with methanolic and aqueous hydrogen chloride, and, after neutralisation with silver carbonate, the hydrolysate was examined on the chromatogram. Quantitative estimation (Hirst, Hough, and Jones, *loc. cit.*) revealed the presence of 2 : 3 : 4 : 6-tetra-*O*-methyl- (R_G 1·0, 5·5%), 2 : 3 : 6-tri-*O*-methyl- (R_G 0·84, 86·1%), di-*O*-methyl- (R_G 0·65, 0·58, 8·4%), and traces of mono-*O*-methyl-glucose and glucose. This result indicated the presence of one non-reducing terminal group per 18—19 glucose residues.

Methylated amylopectin (3·32 g.) was hydrolysed successively with methanolic hydrogen chloride (200 c.c.; 1%) for 5 hr. and with hydrochloric acid (300 c.c.; 0·5N) for 5 hr. The hydrolysate was neutralised with silver carbonate and de-ionised with Amberlite resins IR-100 and IR-4B, and the solution was concentrated to a syrup (3·35 g.). The syrup was fractionated on cellulose (64·3 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later, 1 : 1) saturated with water, and butan-1-ol partly saturated with water, as eluants to give four fractions.

Fraction 1. The syrup (329 mg.) was examined on the chromatogram and showed only 2 : 3 : 4 : 6-tetra-*O*-methylglucose, but hypoiodite oxidation indicated only 49% of aldohexose. A sample (10 mg.) was rehydrolysed and chromatographic examination of the hydrolysate showed that 2 : 3 : 6-tri-*O*-methylglucose was also present. The syrup (310 mg.) was rehydrolysed with hydrochloric acid (20 c.c.; 1%) on the water-bath for 5 hr. After neutralisation with silver carbonate, the resulting syrup (290 mg.) was fractionated on filter sheets, using solvent C, to give fractions 1*a* (105 mg.) and 1*b* (112 mg.). Fraction 1*a* crystallised and after two recrystallisations from light petroleum (b. p. 40—60°) had m. p. and mixed m. p. (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose) 86—88° and $[\alpha]_D^{18}$ +98° → +83° (equil.) (*c*, 0·4 in H_2O) (Found: C, 51·2; H, 8·5; OMe, 52·0. Calc. for $C_{10}H_{20}O_6$: C, 51·2; H, 8·5; OMe, 52·5%). The derived 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine had m. p. and mixed m. p. 136—138° (Found: N, 4·3; OMe, 39·4. Calc. for $C_{16}H_{25}O_5N$: N, 4·5; OMe, 39·9%). Fraction 1*b* crystallised and after two recrystallisations from dry ether had m. p. and mixed m. p. (with 2 : 3 : 6-tri-*O*-methyl-D-glucose) 115—117°, $[\alpha]_D^{18}$ +88° → +68° (equil.) (*c*, 0·4 in H_2O) and $[\alpha]_D^{18}$ +67° → −35° (10 hr., const.) (*c*, 0·4 in methanolic 2% hydrogen chloride) (Found: OMe, 41·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). From the above results the amount of tetra-*O*-methyl-D-glucose was calculated to be 160 ± 8 mg., corresponding to one non-reducing terminal group per 18—19 glucose residues.

Fraction 2. The syrup (2·31 g.) crystallised and after two recrystallisations from dry ether had m. p. and mixed m. p. (with 2 : 3 : 6-tri-*O*-methyl-D-glucose) 115—117°, $[\alpha]_D^{18}$ +90° → +66° (equil.) (*c*, 1·0 in H_2O) and $[\alpha]_D^{18}$ +67° → −34° (10 hr., const.) (*c*, 1·0 in methanolic 2% hydrogen chloride) (Found: OMe, 41·5. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction 3. Chromatographic examination of the syrup (160 mg.) showed the presence of 2 : 3 : 6-tri-*O*-methyl- and 2 : 3-di-*O*-methyl-glucose. Separation on filter sheets with solvent B gave fractions 3*a* (28 mg.) and 3*b* (95 mg.). Fraction 3*a* was identified as 2 : 3 : 6-tri-*O*-methyl-D-glucose, m. p. and mixed m. p. 114—116°. Fraction 3*b* was a syrup which did not crystallise, but was chromatographically homogeneous (R_G 0·64 in solvent B) and had $[\alpha]_D^{18}$ +106° → +68° (equil.) (*c*, 0·4 in H_2O) (Found: OMe, 29·2. Calc. for $C_8H_{16}O_6$: OMe, 29·7%). The sugar was identified as 2 : 3-di-*O*-methyl-D-glucose by conversion into 2 : 3-di-*O*-methyl-D-gluconophenylhydrazide, m. p. 160—162° (Found: OMe, 19·2. Calc. for $C_{14}H_{22}O_6N_2$: OMe, 19·7%).

Fraction 4. The syrup (70 mg.), which did not crystallise, had $[\alpha]_D^{18}$ +74° → +58° (equil.) (*c*, 0·5 in H_2O) and $[\alpha]_D^{18}$ +60° → −10° (8 hr., const.) (*c*, 0·5 in methanolic hydrogen chloride) (Found: OMe, 29·0. Calc. for $C_8H_{16}O_6$: OMe, 29·7%). Chromatographic examination suggested the presence of 2 : 6- and/or 3 : 6-di-*O*-methyl-D-glucose (R_G 0·58 in solvent B). The syrup (35 mg.) was converted into the methyl glycosides and treated with sodium metaperiodate by Bell's method (*J.*, 1948, 992). 0·53 Mole of periodate was consumed per $C_9H_{18}O_6$ unit, indicating the presence of 53% of 2 : 6-di-*O*-methyl-D-glucose in the fraction. After destruction of excess of periodate, the chloroform-soluble extract was hydrolysed and chromatographic examination showed a single sugar (R_G 0·59 in solvent B) corresponding to 3 : 6-di-*O*-methyl-D-glucose. Hypoiodite oxidation showed 14·5 mg. of the sugar to be present, corresponding to 41% of the fraction.

Determination of Glucose Residues in the Amylopectin unattacked by Periodate.—Amylopectin (0·985 g.) was dissolved in water (80 ml.) containing potassium chloride (5 g.), and 0·3M-sodium metaperiodate (30 ml.) was added. The mixture was shaken in the dark for 10 days. After removal of periodate and other ions the oxidised polysaccharide was dissolved in 0·2N-sodium hydroxide (30 ml.), and sodium borohydride (0·25 g.) was added. After being kept overnight the solution was neutralised with acetic acid, dialysed, and taken to dryness. The resulting polyol was hydrolysed and the glucose formed was estimated by quantitative paper chromatography. The quantity of glucose found (7·3 mg.) showed that 0·63% of the glucose residues in the amylopectin were unattacked by periodate.

β-*Amylolysis of the Amylopectin.*—Amylopectin (24·3 mg.) was incubated at 73° with 0·2M-acetate buffer (pH 4·6; 10 c.c.), distilled water (39 c.c.), and crystalline sweet-potato β-amylase solution (1 c.c.) (kindly supplied by Dr. D. J. Manners). Aliquot portions were removed at intervals for determination of reducing power (as maltose) by means of the Shaffer–Somogyi reagent 60 as modified by Hanes and Cattle (*Proc. Roy. Soc.*, 1938, *B*, **125**, 387). After 4 hr. the conversion into maltose was complete at 44%.

Isolation of Malted-barley Amylopectin β-*Limit Dextrin.*—Amylopectin (0·624 g.) was dissolved in water (305 c.c.), and 0·2M-acetate buffer (pH 4·6; 100 c.c.) and β-amylase solution [25 c.c.; prepared from barley flour by Northcote's method (*Biochem. J.*, 1953, **53**, 348)] were added.

After incubation for 48 hr. at 37° the conversion into maltose was complete at 47%. The solution was boiled for 10 min. to inactivate the enzyme, and coagulated protein was removed by filtration. The solution was dialysed for 2 days, and the dextrin was precipitated with ethanol, redissolved in water (50 c.c.), and freeze-dried (yield, 0·274 g.) (Found : N, 0·9%). The dextrin (0·244 g.) was dispersed in water (100 c.c.) and shaken for 1 hr. with butanol (100 c.c.). The brown layer formed at the interface was removed, and the dextrin was precipitated with ethanol, dispersed in water, and freeze-dried (yield, 0·208 g.) (Found : N, 0·05%). The dextrin had $[\alpha]_D^{18}$ +149° (*c*, 0·5 in N-NaOH). Periodate oxidation of a sample gave formic acid corresponding to one non-reducing group per 8—9 glucose residues.

Examination of malted-barley amylose.

Amylose B had $[\alpha]_D^{16}$ +200° (*c*, 0·5 in H_2O), $[\alpha]_D^{15}$ +143° (*c*, 0·5 in N-NaOH), and $[\alpha]_D^{15}$ +205° (*c*, 0·5 in 30% $HClO_4$), and a blue value of 1·25. Potentiometric titration showed that the amylose had a maximum iodine-binding power of 19·2%. Hydrolysis of a sample gave only glucose (95·0%).

Periodate oxidation of the amylose gave one mol. of formic acid per 110—115 glucose residues. If one assumes the release of two mols. of formic acid from the reducing end-group and one mol. from the non-reducing end-group, this value corresponded to a chain length of 330—345 glucose residues. Hydrolysis of the periodate-oxidised amylose showed that there were no unattacked glucose residues.

Methylation of the Amylose.—Malt amylose (7·5 g.) was partly methylated by being kept at 0° in an ethereal solution of diazomethane (cf. Hough and Jones, *Chem. and Ind.*, 1952, 380). When the ethereal solution became colourless it was removed by decantation and fresh ethereal diazomethane was added. After 34 weeks the partly methylated amylose was isolated (Found : OMe, 20·2%). The polysaccharide was then methylated five times with methyl sulphate and sodium hydroxide solution under nitrogen at room temperature, and the product (7·56 g.; OMe, 44·2%) was fractionated in boiling chloroform–light petroleum (b. p. 60—80°) mixtures, to give a main fraction (6·17 g.), $[\alpha]_D^{17}$ +206° (*c*, 1·0 in $CHCl_3$), $\eta_{sp.}/c$, 0·82 (*c*, 0·4 in *m*-cresol) (OMe, 44·8%).

Hydrolysis of Methylated Amylose and Separation of Methylated Sugars.—A sample of the methylated amylose (50 mg.) was hydrolysed and chromatographic examination of the hydrolysate showed the presence of 2 : 3 : 6-tri-*O*-methylglucose together with small quantities of di-*O*-methylglucose. The major part of the methylated amylose (4·25 g.) was hydrolysed as described for methylated amylopectin, and the resulting mixture of sugars (4·21 g.) was fractionated on cellulose in the usual way to give three fractions.

Fraction 1. Chromatographic examination of the syrup (27 mg.) showed only 2 : 3 : 4 : 6-tetra-*O*-methylglucose, but hypoiodite oxidation indicated only 47% of aldohexose. A sample (1 mg.) was rehydrolysed and chromatographic examination of the hydrolysate showed that 2 : 3 : 6-tri-*O*-methylglucose was also present. The syrup (22 mg.) was rehydrolysed with 1% hydrochloric acid (10 c.c.) on the water-bath for 5 hr. After neutralisation with silver carbonate, the resulting syrup (21 mg.) was fractionated on filter sheets with solvent C, to give fractions 1*a* (10·5 mg.) and 1*b* (9 mg.). Fraction 1*a* crystallised and had m. p. and mixed m. p. (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose) 78—80°. Fraction 1*b* which did not crystallise, had R_G 0·82 in solvent B, corresponding to 2 : 3 : 6-tri-*O*-methyl-D-glucose, but was not examined further. From the above results the amount of tetra-*O*-methyl-D-glucose was calculated to be 12·8 ± 0·4 mg., corresponding to one non-reducing terminal group per 310 ± 20 glucose residues.

Fraction 2. The syrup (3·58 g.) crystallised and after two recrystallisations from dry ether had m. p. and mixed m. p. (with 2 : 3 : 6-tri-*O*-methyl-D-glucose) 115—117°, $[\alpha]_D^{18}$ +94° → +70° (equil.) (*c*, 1·0 in H_2O) and $[\alpha]_D^{18}$ +63° → −34° (8 hr., const.) (*c*, 0·95 in methanolic 2% hydrogen chloride) (Found : OMe, 41·4. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction 3. The syrup (51 mg.), which did not crystallise, had $[\alpha]_D^{18}$ +58° → −11° (8 hr., const.) (*c*, 0·4 in methanolic 2% hydrogen chloride) (Found : OMe, 29·0. Calc. for $C_8H_{16}O_6$: OMe, 29·7%). Chromatographic examination showed the presence of sugars corresponding to 2 : 3-di-*O*-methyl-D-glucose (R_G 0·59 in solvent B) and to 2 : 6- and/or 3 : 6-di-*O*-methyl-D-glucose (R_G 0·53). Quantitative determination (Hirst, Hough, and Jones, *loc. cit.*) indicated the presence of 7·5% of 2 : 3-di-*O*-methyl-D-glucose (4 mg.). Periodate oxidation of the derived methyl glycosides (Bell, *loc. cit.*) showed that 79% of the fraction was 2 : 6-di-*O*-methyl-D-glucose. The periodate-oxidised methyl glycosides were hydrolysed and chromatographic examination of the hydrolysate showed the presence of 2 : 3- and 3 : 6-di-*O*-methylglucose.

Examination of barley starch and barley amylopectin.

In the following experiments the barley used corresponded to that from which the malted barley examined above was derived (*i.e.*, a mixture of Plumage Archer and Spratt Archer varieties).

Isolation and Purification of Barley Starch.—Crude barley starch (52 g.) was prepared in a manner similar to that described for malted barley starch, and the purification was carried out as for malt starch A. The pure barley starch (5 g.) had $[\alpha]_D^{18}$ +154° (*c*, 1·0 in N-NaOH) (Found : N, 0·02%). Potentiometric titration showed the presence of 22·6% of amylose. Periodate oxidation of a sample of the starch gave formic acid corresponding to one terminal non-reducing group per 32 glucose residues. The value corresponded to a chain length of 24 glucose residues in the amylopectin fraction.

Preparation of Barley Amylopectin.—Barley amylopectin was prepared from crude barley starch as described for malt amylopectin B. The amylopectin had $[\alpha]_D^{18}$ +148° (*c*, 1·2 in N-NaOH) (Found : N, 0·05%). The formic acid produced on periodate oxidation corresponded to an average chain length of 26 glucose residues. Potentiometric iodine titration showed the presence of 0·70% of amylose.

β-Amylolysis of Barley Amylopectin and Isolation of the β-Limit Dextrin.—Barley amylopectin was incubated with barley β-amylase, and after 46 hr. the conversion into maltose was complete at 59%. The β-limit dextrin was isolated as described for the malt dextrin. Barley amylopectin β-limit dextrin had $[\alpha]_D^{18}$ +153° (*c*, 0·5 in N-NaOH) (Found : N, 0·04%). Periodate oxidation of a sample gave formic acid corresponding to an average chain length of 9—10 glucose residues.

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THE 1:4-LACTONE OF (±) 2:4-DIHYDROXY-2-HYDROXYMETHYLBUTANOIC ACID, A NEW SACCHARINOLACTONE

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The common structural feature of the hemicelluloses of the xylan group is a backbone of 1 : 4-linked β-D-xylopyranose residues, the xylans from various plants or from different parts of the same plant differing in the number and nature of the sugar residues linked as side-chains.[1] In many cases the extraction of these polysaccharides from lignified tissues requires the use of alkaline solutions, and it is possible that many structural investigations have been carried out on materials which have undergone alkaline degradation[2] during their isolation. In order to facilitate a study of the alkaline degradation of xylans, we have synthesized xylo*iso*saccharinolactone (the 1 : 4-lactone of (±) 2 : 4-dihydroxy-2-hydroxymethylbutanoic acid) which would be expected to be formed from the alkaline degradation of reducing carbohydrates containing 1 : 4-linked D-xylopyranose residues. The saccharinolactone isolated from the action of alkali on xylobiose (4-*O*-β-D-xylopyranosyl-D-xylose) has been shown to be identical with this synthetic lactone.

1 : 4-Diacetoxybutan-2-one, prepared by the hydration of butyne-1 : 4-diol diacetate,[3] was converted into the cyanohydrin via the bisulphite addition compound; hydrolysis of the crude cyanohydrin, which was accompanied by removal of the acetyl groups and lactonization, yielded the 1 : 4-lactone of (±) 2 : 4-dihydroxy-2-hydroxymethylbutanoic acid, m.p. 95·5–96·5°. The structure of the lactone was confirmed by the isolation of formaldehyde and α-tetronic acid[4] on periodate oxidation.

Xylobiose, prepared from the partial hydrolysis of esparto xylan,[5] was treated with oxygen-free lime water for 144 hr. The solution was deionized with Amberlite resin IR-120(H), and after taking to dryness, the resulting syrup was fractionated on filter sheets using as eluant ethyl acetate-acetic acid-water (10 : 1·3 : 1). Extraction of the appropriate sections of the sheets with water gave the *iso*saccharinolactone, m.p. and mixed m.p. (with the synthetic lactone) 95·5–96·5°. It is probable that this saccharinic acid is the same as that isolated by Whistler and Corbett[6] from the action of alkali on the partial hydrolysis products of corn cob xylan; since the structure was indicated by its reduction to methylethylacetic acid.

It is evident from these investigations that reducing saccharides of the xylan group are susceptible to degradation in alkaline solution. It is of interest that Whistler and Corbett report that corn cob xylan is not degraded in alkaline solution, a fact which they attribute to the oxidation of the reducing group of the molecule to the corresponding glyconic acid during the previous treatment of the corn cobs with chlorous acid to remove lignin. The results of a more detailed examination of the nature and extent of the alkaline degradation of xylobiose and of hemicelluloses of the xylan group will be reported elsewhere in due course.

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137. *Studies on Degraded Esparto Cellulose.*

By G. O. Aspinall and W. B. Fordyce.

Degraded esparto cellulose capable of peptisation into a colloidal solution in water has been prepared. Methylation end-group assay showed that the degraded cellulose had an average chain length of 77 ± 3 glucose residues, and a similar value was obtained from periodate oxidation. The chemical accessibility of esparto cellulose, before and after degradation, has been investigated by acid hydrolysis and water sorption methods.

The heterogeneous degradation of cellulose by hot dilute (*ca.* 10%) mineral acid causes severe depolymerisation usually accompanied by destruction of the characteristic fibrous structure. After a rapid initial decrease, the degree of polymerisation tends to reach a limiting value of the order of 50—200 with the formation of fragments relatively resistant to further attack under the conditions of hydrolysis. These resistant fragments, whose exact size depends on the source and previous treatment of the original cellulose,[1] are considered to arise from preferential hydrolysis of the amorphous regions of the cellulose fibre and to represent the more highly ordered or crystalline regions. Indeed, methods for the determination of the degree of crystallinity of celluloses are based on the relative ease of accessibility to chemical attack of the amorphous regions.[2] Rånby [3] has shown that some of these resistant fragments (or cellulose micelles) will peptise to form aqueous sols in the pH range 3·5—9·5 and in the absence of electrolytes. Electron-microscopic measurements showed that the dimensions of the micelles (*ca.* 500 × 80 Å) were similar to those of the ordered regions or crystallites of the long filamentary fibrils obtained by ultrasonic treatment of cellulose fibres; [3,4] the lengths of the micelles corresponded to the values for the degrees of polymerisation of the derived cellulose nitrates measured viscometrically. These cellulose micelles, however, had not been examined by chemical methods, and this paper describes the results of an investigation of such a degraded cellulose prepared from esparto grass (*Stipa tenacissima* L.).

Esparto cellulose, obtained by exhaustive extraction of the delignified grass with dilute alkali to remove hemicelluloses, was hydrolysed with hot dilute sulphuric acid, and cellulose sols were obtained by repeated washing of the resulting hydrocellulose with water. Peptisation occurred after the fourth or fifth washing, and the colloidal particles, which showed the hydrophobic properties described by Rånby,[3] coagulated in the presence of a small amount of added electrolyte. The degraded esparto cellulose was methylated with rigorous exclusion of oxygen [5] and the quantity of tetra-*O*-methyl-D-glucose isolated from the hydrolysis of the methylated cellulose corresponded to one terminal non-reducing group per 77 ± 3 glucose residues. Estimation of the formic acid produced on periodate oxidation [6] indicated an average chain length of 69—72, a figure in reasonable agreement with the value obtained from the methylation studies.

It is interesting that chromatographic evidence was obtained for the presence of small quantities of xylose in the hydrolysate from the degraded cellulose and of small quantities of 2 : 3 : 4-tri-*O*-methyl and 2-*O*-methyl xyloses in the hydrolysate from the methylated cellulose. These observations provide further evidence for the extremely close association between cellulose and hemicelluloses in the plant.[7] It would be premature, however, to speculate whether this association is purely physical or whether chemical linkages exist between the two components.

Determinations of chemical accessibility, by two methods, were carried out on three cellulose samples: (1) the original esparto cellulose, (2) the "colloidal" degraded esparto cellulose (referred to as degraded esparto cellulose), and (3) the residual insoluble degraded esparto cellulose which did not undergo peptisation during the preparation of the "colloidal" cellulose (hereinafter called esparto hydrocellulose). In the first method, Nickerson's hydrolytic oxidation procedure [8] was followed, and the carbon dioxide liberated when each cellulose was digested in a boiling solution of ferric chloride and hydrochloric

acid was measured gravimetrically. The carbon dioxide evolved from an equivalent quantity of glucose was measured concurrently. The results were calculated by Nickerson's formula [8] on the assumption that the rapid initial hydrolysis was due to attack in the easily accessible or amorphous regions, whilst the subsequent slow hydrolysis was due to attack in the more highly ordered regions, which are penetrated by chemical reagents only with difficulty. Fig. 1 shows the evolution of carbon dioxide from glucose and from the three cellulose samples, and Fig. 2 shows the percentage of each cellulose hydrolysed plotted against time. The sharp change in rate of hydrolysis of the celluloses over the first 2 hr. is brought out more clearly when the rate is plotted against time (Fig. 3). The rapid initial hydrolysis is complete after 2—3 hr. and from the extrapolation of the percentage hydrolysis–time curves to zero time, values for the chemical accessibility of the celluloses were obtained. The values quoted were obtained from the 2—4 hr. period, and although the shapes of the curves do not permit an exact extrapolation, the relation between the three celluloses is indicated, namely, that the degraded cellulose is slightly less, and the hydrocellulose more, accessible than the original esparto cellulose.

Cellulose	Accessibility (%)	Cellulose (%) hydrolysed after 8 hr.
Esparto cellulose	8·4	21
Esparto hydrocellulose	8·5	34
Degraded esparto cellulose	8·2	19

In the second method, the sorption of water vapour by the cellulose samples was measured and Fig. 4 shows a typical plot of moisture regain against relative humidity. The uptake of water for any cellulose is a function of the accessibility, and calculations from the characteristic sigmoid-shaped isotherms were made by using the theoretical sorption isotherm of Hailwood and Horrobin.[9] Again the results show the degraded esparto cellulose to be less accessible than the original esparto cellulose, whilst the hydrocellulose was slightly more accessible. Although both series of experiments suggest that the degraded " colloidal " cellulose is slightly less accessible than the parent esparto cellulose, as would be expected if hydrolysis of the esparto cellulose preferentially removes the less ordered or amorphous regions, the similarity between the two samples is a more striking feature of the results. It is interesting that Hermans [10] has shown, using the *X*-ray

Cellulose	Regain at 50% relative humidity	Accessibility (%)
Esparto cellulose	6·22	35
Esparto hydrocellulose	6·14	36
Degraded esparto cellulose	5·78	31

diffraction method of Hermans and Weidinger,[11] that colloidal cellulose micelles prepared by Rånby's method [3] had the same degree of crystallinity as the wood pulp from which they were derived. On the other hand, the esparto hydrocellulose was less highly ordered and was attacked 2·5 times more rapidly than the " colloidal " cellulose during the later stages of the hydrolytic oxidation (Fig. 3). As the hydrophobic character of the cellulose sols may be attributed to the high lateral order and high degree of internal hydrogen bonding, the process of peptisation appears to favour the separation of the highly ordered particles from the less highly ordered hydrocellulose.

The results of this investigation show that the cellulose sols from the hydrolysis of esparto cellulose contain particles of the same order of magnitude as those isolated by Rånby [3] from a number of sources. The average chain length of the degraded esparto cellulose (77 ± 3) is intermediate between those found by Rånby for the degraded cellulose from wood pulps and from the corresponding mercerised wood celluloses. Although these resistant colloidal particles arise from the preferential hydrolysis of the amorphous regions of tle original cellulose, it is unlikely that they can be identified with the crystalline regions. On the one hand, it is not known how far the crystallite size may decrease during the hydrolysis. On the other hand, there is evidence that as chain scission occurs a rapid crystallisation of the less ordered regions takes place causing an increase in size of the crystallites.[12–14] The fact that the isolated colloidal particles show little increase in degree of crystallinity (or correspondingly little decrease in accessibility) suggests that

crystallite size, determining as it does the proportion of accessible glucose residues in the surface layers of the cellulose particles, may be a more important factor in accessibility determinations than the geometric order prevailing throughout the interior of the crystallite.

FIG. 1. *Evolution of carbon dioxide from (I) glucose, (II) esparto hydrocellulose, (III) esparto cellulose, and (IV) degraded esparto cellulose in boiling* $FeCl_3$–HCl.

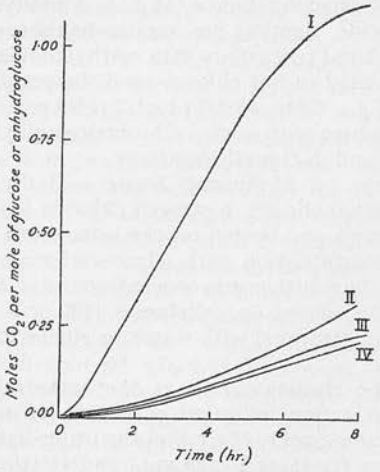


FIG. 2. *Hydrolysis–time curves for (I) esparto hydrocellulose, (II) esparto cellulose, and (III) degraded esparto cellulose.*

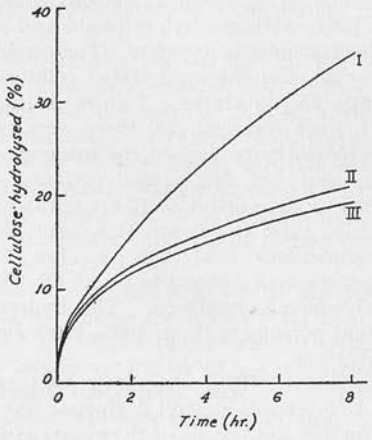


FIG. 3. *Rates of hydrolysis in* $FeCl_3$–HCl *of (I) esparto hydrocellulose, (II) esparto cellulose, and (III) degraded esparto cellulose.*

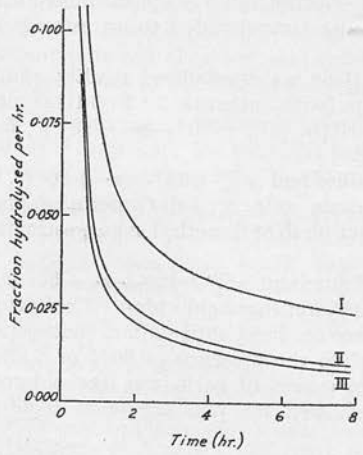
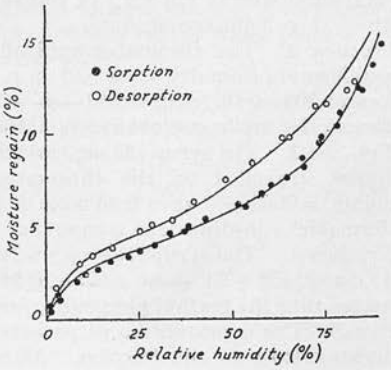


FIG. 4. *Typical water-vapour sorption–desorption isotherm for degraded esparto cellulose.*



EXPERIMENTAL

The following solvents (v/v) were used to separate the sugars and their derivatives: (*A*) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3; top layer), (*B*) butan-1-ol–ethanol–water (4 : 1 : 5; top layer), and (*C*) benzene–ethanol–water (169 : 49 : 15; top layer).

Preparation of Degraded Esparto Cellulose.—Esparto holocellulose was prepared from the grass as described by Chanda, Hirst, Jones, and Percival.[15] The holocellulose (100 g.) was extracted first with cold 4% sodium hydroxide solution (2 l.) for 24 hr. and then hot 6% sodium hydroxide solution (2 l.) for 24 hr. under a steam pressure of 40—45 lb. per sq. in., washed with water until free from alkali, and dried with ethanol to give esparto cellulose (70 g.).

Cellulose sols were prepared in a manner similar to that described by Rånby.[3] Esparto

cellulose (30 g.) was heated at 100° with 10% sulphuric acid (300 c.c.) for 1—8 hr. The degraded cellulose was separated at the centrifuge and washed repeatedly with water (batches of 800 c.c.). Peptisation occurred at the 4th or 5th washing (pH 4—5) and continued until about the 10th washing. The cellulose sols were separated from fibrous material by centrifugation at 1500 r.p.m. and the colloidal cellulose was coagulated by the addition of sodium chloride (0·5 g. per l.). The light flocculent precipitate was separated at the centrifuge, washed twice with water (200 c.c.) to remove the electrolyte, and freeze-dried. The degraded cellulose was obtained in an average concentration of 2 g. per l. Chromatography of the hydrolysate (Monier-Williams) showed the presence of glucose and a trace of xylose.

Methylation of Degraded Esparto Cellulose.—Degraded esparto cellulose (11 g.) was methylated fifteen times with methyl sulphate and sodium hydroxide, rigorous precautions being used to exclude atmospheric oxygen.[5] The product was methylated twice more with methyl iodide and silver oxide, and the methylated cellulose was fractionated in hot chloroform–light petroleum (b. p. 60—65°) mixtures. Two main fractions, 1 (0·85 g.; OMe, 43·4%) and 2 (1·95 g.; OMe, 42·9%), were obtained and these were combined for subsequent work. Chromatography of a sample hydrolysate showed the presence of tetra-, tri-, and di-*O*-methylglucoses.

Hydrolysis of Methylated Cellulose and Separation of Methylated Sugars.—Methylated degraded esparto cellulose (2·6 g.) was heated with methanolic 3% hydrogen chloride (35 c.c.) in a sealed tube at 96° for 24 hr. and the resulting syrup was heated on the water-bath with 0·5N-hydrochloric acid (160 c.c.) for 16 hr. After neutralisation with silver carbonate and deionisation with Amberlite resins IR-100 and IR-4B, the solution was concentrated to a syrup (2·5 g.) which crystallised. The hydrolysate was fractionated on cellulose [16] (100 × 3 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water, as eluant to give four fractions.

Fraction 1. The syrup (43 mg.) travelled on the chromatogram at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose but hypoiodite oxidation indicated only 61% of aldose. After further hydrolysis of the syrup with 0·5N-hydrochloric acid (25 c.c.) on the water-bath for 8 hr., the product was separated on filter sheets to give fractions 1*a* (25 mg.) and 1*b* (10 mg.). Fraction 1*a* crystallised and had m. p. and mixed m. p. (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose) 89—94° $\{[\alpha]_D^{18} + 100° \longrightarrow + 80°$ (*c*, 0·09 in $H_2O)\}$. The derived 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine had m. p. and mixed m. p. 125—130°. Fraction 1*b* travelled on the chromatogram at the same rate as 2 : 3 : 6-tri-*O*-methyl-D-glucose and was not examined further. The corrected weight (29 mg.) of tetra-*O*-methyl-D-glucose corresponded to an average chain length of 77 ± 3 glucose residues.

Fraction 2. The chromatographically pure syrup (1·88 g.) crystallised readily and after recrystallisation from dry ether had m. p. and mixed m. p. (with authentic 2 : 3 : 6-tri-*O*-methyl-D-glucose) 108—113°, $[\alpha]_D^{18} + 85° \longrightarrow + 63°$ (*c*, 0·25 in H_2O), $[\alpha]_D^{18} + 70° \longrightarrow - 38°$ (*c*, 0·58 in methanolic 1% hydrogen chloride); OMe, 42·4%.

Fraction 3. The syrup (35 mg.) which did not crystallise had $[\alpha]_D^{18} + 95° \longrightarrow + 60°$ (*c*, 0·3 in H_2O) and travelled on the chromatogram at the same rate 2 : 3-di-*O*-methyl-D-glucose. Periodate oxidation [17] gave 0·86 mole of formaldehyde per mole of dimethyl sugar, estimated as the formaldehyde–dimedone compound.

Fraction 4. The syrup (194 mg.) which did not crystallise had $[\alpha]_D^{18} + 72° \longrightarrow + 55°$ (*c*, 0·15 in H_2O) and $[\alpha]_D^{18} + 73° \longrightarrow - 50°$ (*c*, 0·26 in methanolic 1% hydrogen chloride). The syrup was converted into the methyl glycosides, and the product was oxidised with sodium metaperiodate solution.[17] The consumption of periodate corresponded to the presence of 66% of 2 : 6-di-*O*-methyl-D-glucose in the fraction. After destruction of excess of periodate, the chloroform-soluble extract was hydrolysed and chromatography showed the presence of a single sugar corresponding to 3 : 6-di-*O*-methyl-D-glucose.

In addition to the major components described, chromatographic evidence was obtained for the presence of traces of 2 : 3 : 4-tri-*O*-methyl- and 2-*O*-methyl-xylose (R_G 0·97 and 0·05 in solvent *C*).

Periodate Oxidation of Degraded Esparto Cellulose.—Oxidation of the cellulose (100 mg. batches) with potassium metaperiodate solution by the method of Halsall, Hirst, and Jones [6] yielded a practically constant amount of formic acid after 49 hr., corresponding to 1 mole per 23—24 glucose residues. The production of 1 mol. of formic acid per non-reducing end-group and 2 mols. per reducing end-group being assumed, this value corresponds to an average chain length of 69—72. A sample of degraded cellulose, prepared in a similar manner from Whatman No. 1 filter paper, yielded, on periodate oxidation, formic acid corresponding to an average chain length of 91—94.

Determination of Chemical Accessibility by Nickerson's Method.—The apparatus used was essentially that described by Conrad and Scroggie,[18] in which the carbon dioxide evolved was determined gravimetrically in absorption tubes packed with " Sofnolite " absorbent (15 g.) and magnesium perchlorate (10 g.). To ensure greater uniformity in the conditions under which cellulose and glucose were subjected to the oxidative hydrolysis the two reaction flasks were heated in the same bath at 132° ± 0·5°, and equal flow-rates of the air currents passing through the parallel reaction vessels were maintained.

Cellulose samples (1·5 g.) were heated in a boiling ferric chloride–hydrochloric acid mixture (150 c.c.; 0·6M; 2·4N), the absorption tubes being weighed periodically. The carbon dioxide evolved from samples of glucose (1·3 g.) treated under the same conditions in the parallel reaction flask was determined concurrently.

By means of Nickerson's formula [8] the experimental results (shown in Fig. 1 as carbon dioxide–time data) were converted into percentage hydrolysis–time curves (Fig. 2), from which the accessibilities were obtained by extrapolation to zero time through the 2—4 hr. period.

Determination of Accessibility by Water Sorption Measurements.—Water-vapour sorption and desorption measurements were made with a simple type of McBain–Baker sorption balance.[19] Cellulose samples under investigation were placed in a glass bucket suspended from a glass spring (sensitivity 14·42 cm./g.). The sorption chamber was connected to a high-vacuum pump through a manometer and a drying-tube (magnesium perchlorate), and a bulb containing water as the source of vapour was directly connected to the sorption chamber.

Zero readings were taken after complete evacuation of the system and after the source of water vapour had been completely degassed. The cellulose sample (*ca.* 0·15 g.) was then placed in the bucket and the system was evacuated (*ca.* 36 hr.) until the cellulose was thoroughly dry (as indicated by minimum extension of the spring). Small quantities of water vapour were then admitted, and after equilibrium had been established, the vapour pressure was measured directly by the change in the manometer levels and the moisture regain was calculated from the extension of the spring. The process was repeated until the maximum equilibrium pressure was reached. Desorption measurements were made by following the changes in water-vapour pressure and moisture regain during the gradual evacuation of the system. A typical plot of sorption–desorption data is given in Fig. 4 where moisture regain is plotted against relative humidity. Calculations of the accessibilities of the cellulose samples were made by means of the theoretical isotherm of Hailwood and Horrobin.[9] The best curves were obtained from the experimental data by the application of the method of least squares and Fig. 4 shows the close agreement between observed and calculated values for moisture regain when plotted against relative humidity in the case of the degraded esparto cellulose.

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206. *Plant Gums of the Genus* Khaya. *The Structure of* Khaya grandifolia *Gum.*

By G. O. Aspinall, E. L. Hirst, and N. K. Matheson.

Khaya grandifolia gum has been shown to be composed of residues of D-galactose, L-rhamnose, D-galacturonic acid, and 4-*O*-methyl-D-glucuronic acid, with traces of L-arabinose. Hydrolysis of the methylated gum indicated the presence therein of residues of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 2 : 3 : 6-tri-*O*-methyl-D-galactose, 3-*O*-methyl-L-rhamnose, 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid and 2 : 3-di-*O*-methyl-D-galacturonic acid. On partial hydrolysis the gum gave two aldobiouronic acids, 2-*O*-D-galacturonosyl-L-rhamnose and 4-*O*-(4-*O*-methyl-D-glucuronosyl)-D-galactose, an aldotriouronic acid, *O*-D-galacturonosyl-(1→2)-*O*-L-rhamnose-(1→4)-D-galactose, and other acidic oligosaccharides.

Khaya senegalensis gum has been shown to be composed of the same sugar residues, but in different proportions.

The gum exudate of *Khaya grandifolia*, the West African mahogany tree, has been examined by McIlroy,[1] who obtained evidence for the presence of residues of galactose, rhamnose, and galacturonic acid. The gum, therefore, appeared to be similar to the gums of *Sterculia setigera* [2] and *Cochlospermum gossypium* (" Karaya " gum) [3] rather than to the glucuronic acid-containing gums of the *Acacia* [4-6] and *Prunus* [7-9] genera. A quantity of the gum was kindly placed at our disposal by Dr. R. J. McIlroy and in the present study the main structural features of the gum have been investigated.

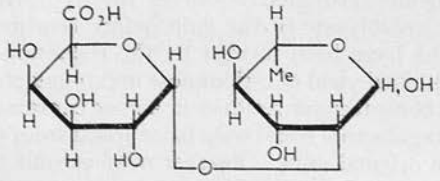
The gum we examined had been purified by dissolution in 4% sodium hydroxide solution, and was further purified by precipitation from acid solution with acetone and then by re-precipitation from aqueous solution with ethanol. The purified gum, in contrast with the crude material, was readily soluble in water. It is possible that the treatment with alkali had removed ester groupings as several plant gums [2,3] are known to occur as partly acetylated polysaccharides. The gum had a uronic anhydride content [10] of 47% (calculated for a substance of equivalent weight 344, 51%) and a low, but significant, methoxyl content (1%).

The gum was extremely resistant to hydrolysis, and even after hydrolysis with 2N-sulphuric acid for 18 hr. at 100° the yield of neutral sugars was low, quantitative estimation [11] indicating the presence of galactose (18%), rhamnose (15%), and arabinose (1%) (results expressed as percentages of anhydro-sugar originally present in the gum). After hydrolysis of the gum with N-sulphuric acid at 100° for 6 hr. L-rhamnose (3·7%), L-arabinose (0·2%), and D-galactose (18·0%) were isolated as crystalline compounds. The incompletely hydrolysed acidic residue (52% of the original weight of gum) was converted into the methyl ester methyl glycoside, reduced with potassium borohydride, and rehydrolysed, the following sugars being then identified : L-rhamnose (10·1%), 4-*O*-methyl-D-glucose (2·0%), D-glucose (trace), and D-galactose (26·6%). It is probable that the trace of glucose arose from the demethylation of 4-*O*-methyl-D-glucose during the hydrolysis. As 4-*O*-methyl-D-glucose was not found in the hydrolysate of the gum before treatment of the gum with potassium borohydride, it must have been formed by the reduction of 4-*O*-methyl-D-glucuronic acid residues. The combined yield of L-rhamnose and D-galactose (58·4%) was greater than could be derived from neutral sugar residues in a gum containing 47% of uronic anhydride, therefore some of the D-galactose could only have arisen from the reduction of D-galacturonic acid residues in the original gum. Further evidence for the presence of galacturonic residues was obtained on nitric acid oxidation of the gum; the yield of mucic acid indicated the presence in the gum of 58% of galactose and/or galacturonic acid residues.

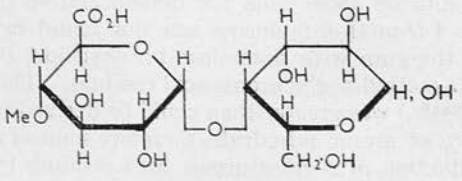
The methylated gum was prepared in the usual way and like the parent gum was extremely resistant to hydrolysis. The hydrolysate was fractionated chromatographically on cellulose,[12] giving 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 2 : 3 : 6-tri-*O*-methyl-D-

galactose, and 3-*O*-methyl-L-rhamnose (all identified as crystalline derivatives), a trace of an unidentified di-*O*-methylgalactose (probably of no structural significance), and a mixture of acidic substances. After attempts to separate the acidic components had failed, the acids were converted into the methyl ester methyl glycosides, which were reduced with lithium aluminium hydride. The reduced glycosides were hydrolysed and the resulting neutral sugars were partitioned on cellulose. Further quantities of 2 : 3 : 6-tri-*O*-methyl-D-galactose and 3-*O*-methyl-L-rhamnose were isolated, and in addition 2 : 3 : 4-tri-*O*-methyl-D-glucose and 2 : 3-di-*O*-methyl-D-galactose were characterised by the formation of crystalline derivatives. As the last two sugars were not present amongst the neutral sugars isolated on direct hydrolysis of the methylated gum it is clear that they were formed by the reduction of 2 : 3 : 4-tri-*O*-methyl-D-glucuronic and 2 : 3-di-*O*-methyl-D-galacturonic acids respectively. No methyl ethers of L-arabinose were detected.

The mixture of acidic substances obtained on partial hydrolysis of the gum was fractionated by stepwise elution from a column of an anion-exchange resin [13] with increasing concentrations of acetic acid, to give a hexuronic acid, a mixture of aldobiouronic acids, an aldotriouronic acid, and other incompletely identified acidic oligosaccharides. The hexuronic acid, isolated in 1·3% yield from the gum, was identified as galacturonic acid by bromine oxidation to mucic acid. The aldobiouronic acid fraction, isolated in 10% yield, was shown by paper ionophoresis [14] to contain two components with slightly different M_G values, although the two substances travelled at the same rate on a paper chromatogram. Hydrolysis of the mixture of acids gave rhamnose, galactose, galacturonic acid, and 4-*O*-methylglucuronic acid, but, after oxidation of the disaccharides with bromine water, rhamnose and galactose could no longer be detected on hydrolysis. The aldobiouronic acids were converted into a mixture of fully methylated disaccharides by reduction of the acidic residues in the methylated aldobiouronic acids with lithium aluminium hydride followed by further methylation of the reduction products. Hydrolysis of the methylated disaccharides then yielded the following sugars, which were identified as crystalline derivatives : 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 3 : 4-di-*O*-methyl-L-rhamnose, and 2 : 3 : 6-tri-*O*-methyl-D-galactose. Although these results indicate the mode of linkage of the sugar residues in the aldobiouronic acids, they do not provide evidence for the combination of sugars. Two observations, however, point to 2-*O*-D-galacturonosyl-L-rhamnose (I) and 4-*O*-(4-*O*-methyl-D-glucuronosyl)-D-galactose (II) as structures of the aldobiouronic acids. First, the two aldobiouronic acids travel on the chromatogram at the same rate, whereas it would be expected from the known rates of movement of the constituent sugars that aldobiouronic acids, in which the sugars were linked in the alternative manner, would travel at different rates. Secondly, only a small difference in the ionophoretic mobilities of the aldobiouronic acids (I) and (II) would be expected, as each disaccharide contains one pair of hydroxyl groups [the 3 : 4-hydroxyl groups of the galacturonosyl residue in (I) and the 1 : 2-hydroxyl groups of the galactose residue in (II)] capable of complex formation with boric acid and the carboxyl group of the acidic residue. On the other hand, the alternative pair of aldobiouronic acids, 4-*O*-D-galacturonosyl-D-galactose and 2-*O*-(4-*O*-methyl-D-glucuronosyl)-L-rhamnose, would be



(I)



(II)

expected to show greatly different ionophoretic mobilities [15] as the former compound would contain two pairs of hydroxyl groups capable of complex formation with boric acid, whereas the latter would contain none. These two aldobiouronic acids have been previously

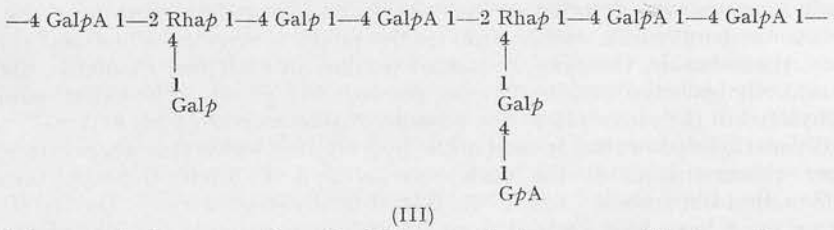
isolated from the hydrolysis of plant gums, namely, 2-*O*-D-galacturonosyl-L-rhamnose from *Sterculia setigera*[2] and *Cochlospermum gossypium*[3] gums, and 4-*O*-(4-*O*-methyl-D-glucuronosyl)-D-galactose from gum myrrh.[16]

An aldotriouronic acid (equivalent weight 490) was isolated in 7·0% yield, and gave on hydrolysis galactose and rhamnose. After oxidation of the trisaccharide with bromine water only rhamnose was detected on hydrolysis, and after reduction of the acidic residue with potassium borohydride and hydrolysis the products were galactose and rhamnose. The acidic trisaccharide, therefore, contained residues of galactose, rhamnose, and galacturonic acid, the galactose residue forming the reducing group. The aldotriouronic acid was methylated in the same way as the mixture of aldobiouronic acids and was converted into a fully methylated neutral trisaccharide, hydrolysis of which gave sugars travelling on the paper chromatogram at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 3 : 4-di-*O*-methyl-L-rhamnose, and 2 : 3 : 6-tri-*O*-methyl-D-galactose. The tri-*O*-methyl-D-galactose must have been derived from the reducing galactose residue linked through position 4, therefore the tetra-*O*-methyl-D-galactose could only have been formed from a galacturonic acid residue at the non-reducing end of the molecule. It follows from this evidence that the acidic trisaccharide was *O*-D-galacturonosyl-(1→2)-*O*-L-rhamnosyl-(1→4)-D-galactose. Other acidic oligosaccharides were obtained in low yield, but although these were all shown to be composed of galactose, rhamnose, and galacturonic acid residues, insufficient quantities were isolated for detailed structural studies. The high proportion of acid residues in one of these fractions (equivalent weight 300) suggested that the gum may contain adjacent galacturonic residues.

The main structural features of *K. grandifolia* gum are now clear from this investigation. From the methylation studies it is evident that end-groups of D-galactose and 4-*O*-methyl-D-glucuronic acid occur in the gum whilst L-rhamnose residues provide the only branching points in the molecule and 1 : 4-linked D-galacturonic acid residues occur in the main chains. The evidence for the presence of the aldobiouronic acid 2-*O*-D-galacturonosyl-L-rhamnose (I) in the products of partial hydrolysis shows that the L-rhamnose residues are linked through positions 1 and 2 in the main chain and that the side chains must be linked through position 4. As glycosiduronic acid linkages are particularly resistant to acid hydrolysis, the evidence that on mild acid hydrolysis of the gum only galactose, together with small amounts of arabinose, was formed and no significant amounts of neutral disaccharides were detected, suggests that the D-galactose end-groups are linked directly to the L-rhamnose residues. On the other hand, the identification of the aldobiouronic acid 4-*O*-(4-*O*-methyl-D-glucuronosyl)-D-galactose (II) on partial acid hydrolysis of the gum shows that the 4-*O*-methyl-D-glucuronic acid end-groups are linked to the main chain through 1 : 4-linked D-galactose residues in two-residue side-chains. The isolation of the aldotriouronic acid, *O*-D-galacturonosyl-(1→2)-*O*-L-rhamnosyl(1→4)-D-galactose, shows that some 1 : 4-linked D-galactose residues are also present in the main chain. In addition, the main chain probably contains adjacent D-galacturonic acid residues, as indicated by the oligosaccharides of high uronic acid content isolated on partial acid hydrolysis of the gum; indeed, the high uronic acid content of the gum itself can only be explained on this basis.

Throughout this investigation the hydrolyses of both the original gum and its methyl ether were accompanied by considerable decomposition and the yields of sugars isolated were low. An attempt to obtain a quantitative estimate of the ratio of methylated sugar residues present in the methylated gum was made by hydrolysing samples of the methylated polysaccharide after reduction of the acidic residues with lithium aluminium hydride, but only an 80% recovery of sugars on hydrolysis was obtained. Consequently only an approximate estimate of the constituent sugars present in the gum can be made. Ultracentrifugal and electrophoretic examinations of the gum (in collaboration with Dr. C. T. Greenwood) indicated the presence of only one molecular species, and all the structural features must therefore be accommodated within a single molecule. Although no unique structure can be put forward for the gum, the repeating unit (III) shown includes all the known types of linkage present in the gum, and the ratio of constituent sugar residues, namely, D-galactose (3 parts), L-rhamnose (2 parts), D-galacturonic acid (4 parts) and 4-*O*-methyl-D-glucuronic acid (1 part), is consistent with the quantitative measurements

made, when allowance is made for the decomposition of sugars during hydrolysis. The ultracentrifugal studies indicated that the gum was of high molecular weight although no accurate value is yet available, and the end-groups of D-galactose and 4-*O*-methyl-D-glucuronic acid must therefore occur in the side-chains rather than as the non-reducing terminal residues of the backbone of the molecule.



(III)

(Gal *p*A = D-galactopyranuronic acid, Gal*p* = D-galactopyranose, Rha*p* = L-rhamnopyranose, and G*p*A = 4-*O*-methyl-D-glucopyranuronic acid.)

These results show that *Khaya grandifolia* gum contains several interesting structural features. The occurrence of two different uronic acid residues within a single molecule is a unique feature of the plant gums so far examined. On previous occasions when two aldobiouronic acids have been isolated from the partial hydrolysis of a plant gum, these have contained the same uronic acid residue linked either to different sugars or to the same sugar through different positions. The gum resembles in some respects those from *Sterculia setigera* [2] and *Cochlospermum gossypium*,[3] which also contain D-galactose, L-rhamnose, and D-galacturonic acid residues, although the present gum contains in addition 4-*O*-methyl-D-glucuronic acid residues. As is also the case for these gums the D-galactose residues in *K. grandifolia* gum are linked through positions 1 and 4, in contrast with damson,[7] cherry,[8] and egg-plum [9] gums, and the gums of the *Acacia* group [5, 6] where the D-galactose residues are linked through positions 1 and 3, and 1 and 6. *K. grandifolia* gum, however, differs from *S. setigera* and *C. gossypium* gums in that L-rhamnose occurs solely as branching points in the central part of the molecular structure and is not also found linked through positions 1 and 2 only. The gum differs also from *S. setigera* gum in that the D-galacturonic acid residues are linked only through positions 1 and 4 in the main chain and do not form the branching points of the gum molecule.

Recent investigations of the gums of the *Acacia* genus [5, 6] have shown that different gums contain the same constituent sugars and in each case the aldobiouronic acid, 6-*O*-D-glucuronosyl-D-galctose, has been isolated from the products of partial hydrolysis, but the proportions in which the sugar residues are present differ markedly. It was of interest therefore to examine another gum of the *Khaya* genus. *Khaya senegalensis* gum was obtained in the natural state as a partially acetylated polysaccharide, and was purified by dissolution in aqueous sodium hydroxide and isolated as a white amorphous powder after three reprecipitations from aqueous solution with ethanol. The purified gum had an equivalent weight of 412 and a methoxyl content of 1·2%. After partial hydrolysis, L-rhamnose (4·0%), L-arabinose (2·1%), and D-galactose (16·1%) were isolated as crystalline compounds. The incompletely hydrolysed acidic residue (37·8%), which had an equivalent weight of 348, liberated further quantities of rhamnose and galactose on extended hydrolysis. Chromatographic examination of the acidic residue indicated a mixture of acidic oligosaccharides similar to that obtained on partial hydrolysis of *K. grandifolia* gum. After conversion into the methyl ester methyl glycoside, reduction with potassium borohydride, and hydrolysis of the reduction product, chromatographic examination of the hydrolysate showed rhamnose, 4-*O*-methylglucose, glucose (trace), and galactose to be present. The methoxyl content of the gum was not high enough for all the uronic acid to be present as 4-*O*-methylglucuronic acid, so it is probable that galacturonic acid is also a constituent sugar of the gum. *K. senegalensis* gum, therefore, contains the same sugar residues as *K. grandifolia* gum, but the proportions of uronic acid and L-arabinose are different. The evidence at present available indicates that in the *Khaya* genus the position in regard to the composition of the various gums is similar to that found for *Acacia* species.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper, with the upper layers of the following solvent systems (v/v): (*A*) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (*B*) butan-1-ol–ethanol–water (4 : 1 : 5); (*C*) butan-1-ol–acetic acid–water (4 : 1 : 5); (*D*) butal-1-ol–acetic acid–water (8 : 2 : 5); (*E*) benzene–ethanol–water (169 : 47 : 15). Paper ionophoresis [14] was carried out in borate buffer at pH 10.

Purification and Properties of Khaya grandifolia *Gum.*—The gum was received from Dr. R. J. McIlroy as a light grey powder which had been reprecipitated with ethanol after dissolution in 4% aqueous sodium hydroxide.[1] The gum acid was precipitated from solution in dilute hydrochloric acid with ethanol and reprecipitated from aqueous solution with acetone, to give a white amorphous powder, $[\alpha]_D^{18}$ +122° (unchanged on further reprecipitation) (*c*, 1·88 in H_2O) [Found: equiv., 344 (by titration); uronic anhydride, 47·2% (by decarboxylation); ash, nil; OMe, 1·0%].

Chromatographic examination of the hydrolysate of the gum after it had been heated with N-sulphuric acid at 100° showed that galactose and arabinose (trace) were released after 0·5 hr. and that rhamnose appeared after 3 hr. The hydrolysis of the gum under milder conditions was also followed (autohydrolysis at 100° for 40 hr., hydrolysis with 0·01N-sulphuric acid at 100° for 40 hr., and hydrolysis with 0·1N-sulphuric acid at 100° for 14 hr.), but in no case were significant amounts of neutral disaccharides detected in addition to galactose and arabinose.

Quantitative estimation, by the method of Hirst and Jones,[11] of the sugars produced on hydrolysis of the gum with N-sulphuric acid at 100° for 6 hr. indicated the presence of the following sugars (calc. as percentage of anhydro-sugar in the original gum): galactose, 16·1; rhamnose, 8·2; arabinose, <1%. After hydrolysis of the gum with 2N-sulphuric acid at 100° for 18 hr. the same sugars were present in the following quantities: galactose, 18·0; rhamnose, 14·7; arabinose, <1%. Oxidation of the gum with nitric acid and estimation of the mucic acid (m. p. and mixed m. p. 216°) formed indicated the presence in the gum of 58% of galactose and/or galacturonic acid residues.

Partial Hydrolysis of the Gum.—The purified gum (10 g.) was heated with N-sulphuric acid (250 c.c.) at 100° for 6 hr., the hydrolysate was neutralised with barium hydroxide, the excess of alkali rapidly destroyed with carbon dioxide, and the filtrate was taken to dryness. The residue was fractionated on cellulose,[12] with butan-1-ol saturated with water plus 10% of ethanol, as the eluant, giving four fractions.

Fraction 1 (0·370 g.) had $[\alpha]_D^{17}$ +9·8° (equil.) (*c*, 4·44 in H_2O) and after recrystallisation from ethanol had m. p. and mixed m. p. (with L-rhamnose hydrate) 95°. Fraction 2 (0·023 g.) had $[\alpha]_D^{17}$ +104° (equil.) and after recrystallisation from methanol had m. p. and mixed m. p. (with L-arabinose) 155°. Fraction 3 (1·80 g.) had $[\alpha]_D^{16}$ +81° (equil.) and after recrystallisation from methanol had m. p. and mixed m. p. (with D-galactose) 164°. Fraction 4, obtained by elution of the cellulose with water, consisted of the barium salts of acidic material (Found: Ba, 17·4%). Barium ions were removed by passage through a column of Amberlite resin IR-120(H) and the solution was freeze-dried to give the acidic fraction (A) (5·2 g.) which was examined later.

Hydrolysis of the Acidic Fraction (A) after Reduction with Potassium Borohydride.—The acidic fraction (A) (4·5 g.) was set aside overnight in methanolic 4% hydrogen chloride and then refluxed for 7 hr. After neutralisation with silver carbonate, the dry residue was dissolved in water (100 c.c.) and the solution was added slowly to a solution of potassium borohydride (2·3 g.) in water (100 c.c.). After 2 hr., the excess of borohydride was destroyed by the addition of dilute acetic acid, and the solution was de-ionised by passage through columns of Amberlite resins IR-120 and IR-4B. After being taken to dryness the residue was hydrolysed by N-sulphuric acid at 100° for 18 hr. Chromatographic examination of the hydrolysate showed rhamnose, 4-*O*-methylglucose, glucose (trace), and galactose, and partition on cellulose, with, as eluant, butan-1-ol saturated with water, gave pure fractions of glucose and galactose, and a mixture of rhamnose and 4-*O*-methylglucose. Most of the rhamnose crystallised on trituration of the mixed fraction with moist butan-1-ol and complete separation of the two components was achieved by further partition on cellulose. Examination of the four fractions confirmed the identity of the sugars. Fraction 1 (0·878 g.) had $[\alpha]_D^{18}$ +10° (equil.) (*c*, 2·35 in H_2O) and after recrystallisation from moist butan-1-ol had m. p. and mixed m. p. (with L-rhamnose hydrate) 95°. Fraction 2 (0·173 g.) had $[\alpha]_D^{20}$ +62° (Found: OMe, 15·5. Calc. for $C_7H_{14}O_6$: OMe, 15·9%), and was identified as 4-*O*-methyl-D-glucose by conversion into the osazone, m. p. 154°, $[\alpha]_D^{19}$ −31°→−14° (equil.) (*c*, 1·11 in H_2O). Fraction 3 (11 mg.) travelled on the chromatogram at the same rate as D-glucose and was destroyed on incubation with glucose-oxidase.

Fraction 4 (2·30 g.) had $[\alpha]_D^{18}$ +81° (equil.) (*c*, 2·34 in H_2O) and after recrystallisation from methanol had m. p. and mixed m. p. (with D-galactose) 163°.

Separation of Acidic Components obtained on Partial Hydrolysis of the Gum.—The acidic fraction (A) (4·0 g.) was absorbed on a column of Amberlite resin IRA-400 (acetate form) (30 × 3·4 cm.; 100 mesh) and the column was eluted with increasing concentrations of dilute acetic acid (0·1—1·0 by stages of 0·1%, 1·0—2·0 by 0·2%, 2·0—5·0 by 0·5%, and 5·0—15·0 by 2·5%). Six main fractions were obtained together with small quantities of complex mixtures which were not examined further.

Fraction 1. The syrup (0·172 g., eluted with 0·2—0·7% acetic acid) contained a main component having R_{Gal} 0·40 (rate of movement relative to galactose in solvent *D*) and M_G 0·65 (rate of movement on the ionophoretogram relative to glucose). Hydrolysis with 2N-sulphuric acid at 100° for 18 hr. gave galactose and rhamnose, but after oxidation with bromine water hydrolysis gave galactose and only a trace of rhamnose. Hydrolysis of the syrup after reduction of the methyl ester methyl glycoside with potassium borohydride gave galactose and rhamnose.

Fraction 2. The syrup (0·731 g., eluted with 0·8—1·0% acetic acid) had $[\alpha]_D^{16}$ +130° (*c*, 1·83 in H_2O). Chromatographic examination suggested the presence of two components, R_{Gal} 0·80, which were incompletely resolved, but complete resolution was achieved on the ionophoretogram (M_G 0·82 and 0·69). Hydrolysis with 2N-sulphuric acid gave rhamnose, galactose, galacturonic acid, and 4-*O*-methylglucuronic acid, but after oxidation with bromine water hydrolysis gave the acidic components only.

A portion of the syrup (0·60 g.) was methylated four times with methyl sulphate and sodium hydroxide, and the product (after extraction of the acidified reaction mixture with chloroform) (0·684 g.) was further methylated with methyl iodide and silver oxide. After removal of the solvent, the syrup was dissolved in dry tetrahydrofuran and added slowly to lithium aluminium hydride (0·30 g.) in tetrahydrofuran. After 3 hr. the excess of lithium aluminium hydride was destroyed by the addition of water, and the solution was acidified and extracted with chloroform. The extract was re-methylated three times with methyl iodide and silver oxide, and the mixture of methylated disaccharides was isolated as a yellow syrup (0·410 g.) (Found: OMe, 52·1. A methylated disaccharide composed of a hexose and a deoxyhexose residue requires OMe, 51·1%, and a methylated disaccharide composed of two hexose residues requires OMe, 54·6%). The methylated sugars were hydrolysed with N-sulphuric acid (10 c.c.) at 100° for 16 hr. and the hydrolysate was separated on cellulose, with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water, as eluant, to give three fractions. Fraction *a* (93 mg.) crystallised and had m. p. and mixed m. p. (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose) 84°, $[\alpha]_D^{17}$ +82° (equil.) (*c*, 1·12 in H_2O) (Found: OMe, 52·8. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). Fraction *b* (200 mg.) contained two sugars having R_G 0·88 and 0·84 in solvent *B*, which were separated on filter sheets with solvent *E* to give fractions *b*(i) (82 mg.) and *b*(ii) (76 mg.). Fraction *b*(i) had $[\alpha]_D^{18}$ +97° (equil.) (*c*, 1·32 in H_2O) and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 194°, $[\alpha]_D^{18}$ −60°→+18° (equil.) (*c*, 0·8 in Me_2CO). Fraction *b*(ii) had $[\alpha]_D^{17}$ +20° (equil.) (*c*, 1·42 in H_2O) (Found: OMe, 32·2. Calc. for $C_8H_{16}O_5$: OMe, 32·3%) and was identified as 3 : 4-di-*O*-methyl-L-rhamnose by conversion into 3 : 4-di-*O*-methyl-L-rhamnono-1 : 5-lactone, m. p. 80°. Fraction *c* (73 mg.) had $[\alpha]_D^{18}$ +88 (equil.) (*c*, 0·5 in H_2O) (Found: OMe, 41·0. Calc. for $C_9H_{18}O_6$: OMe, 41·9%) and was identified as 2 : 3 : 6-tri-*O*-methyl-D-galactose by conversion into 2 : 3 : 6-tri-*O*-methyl-D-galactono-1 : 4-lactone, m. p. and mixed m. p. 96°, $[\alpha]_D^{20}$ −42°→−25° (equil.) (*c*, 0·57 in H_2O).

Fraction 3. The syrup (60 mg., eluted with 1·0—2·0% acetic acid) had R_{Gal} 0·38 and M_G 1·06. Hydrolysis with 2N-sulphuric acid gave rhamnose and galactose, but after oxidation with bromine water hydrolysis gave only rhamnose. Hydrolysis of the syrup after reduction of the methyl ester methyl glycoside with potassium borohydride gave galactose and rhamnose.

Fraction 4. The syrup (0·102 g., eluted with 2·5—3·0% acetic acid) had $[\alpha]_D^{18}$ +30° (*c*, 1·42 in H_2O) and contained a main component, R_{Gal} 0·85 and M_G 1·07, which was identified as D-galacturonic acid by conversion into mucic acid, m. p. and mixed m. p. 220° (decomp.).

Fraction 5. The syrup (0·528 g., eluted with 4·0—12·5% acetic acid) had R_{Gal} 0·36 and M_G 0·79 (Found: equiv., 490). Hydrolysis with 2N-sulphuric acid gave rhamnose and galactose, but after oxidation with bromine water hydrolysis gave only rhamnose. Hydrolysis of the syrup after reduction of the methyl ester methyl glycoside with potassium borohydride gave galactose and rhamnose. A portion of the syrup (0·346 g.) was methylated in the same way as fraction 2 and after reduction of the acidic residues was converted into the fully methylated trisaccharide (0·071 g.). Chromatographic examination of the hydrolysate after it had been heated with N-hydrochloric acid at 100° for 4 hr. showed the presence of sugars travelling at

the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 3 : 4-di-*O*-methyl-L-rhamnose, and 2 : 3 : 6-tri-*O*-methyl-D-galactose in solvents *B* and *E*.

Fraction 6. The syrup (0·65 g., eluted with 12·5—15·0% acetic acid) contained a mixture of substances of R_{Gal} 0·0—0·19 with a main component having M_G 0·93 (Found : equiv., 300). Hydrolysis with 2N-sulphuric acid gave galactose and rhamnose, and the same sugars only were formed on hydrolysis after reduction of the methyl ester methyl glycoside with potassium borohydride.

Methylation of the Gum.—The purified gum (20 g.) was methylated six times with methyl sulphate and sodium hydroxide and, after careful acidification of the reaction mixture and dialysis to remove inorganic ions, the partially methylated gum was converted into the silver salt by treatment of an aqueous solution with silver carbonate and the silver salt was isolated by freeze-drying. The silver salt was suspended in a boiling mixture of methyl iodide (80 c.c.) and methanol (100 c.c.), and silver oxide (15 g.) was added during 2 hr. After removal of insoluble silver salts five further methylations were carried out with methyl iodide and silver oxide, the methylated polysaccharide (10·1 g.), soluble in chloroform–light petroleum (b. p. 60—80°) (1 : 4), was isolated having $[\alpha]_D^{16}$ +53° (*c*, 1·32 in $CHCl_3$) (Found : OMe, 40·2%).

Hydrolysis of the Methylated Gum and Identification of the Neutral Sugars.—The methylated gum (8·0 g.) was dissolved in 2N-sulphuric acid (125 c.c.) and kept at room temperature for 10 days. The solution was then heated at 100° for 20 hr., cooled, and carefully neutralised with barium hydroxide. After carbon dioxide had been bubbled through the mixture to remove excess of hydroxide as barium carbonate, the precipitated barium salts were removed and the solution was taken to dryness. The resulting mixture of sugars was separated on cellulose, with light petroleum (b. p. 100—120°)–butan-1-ol (6 : 4), saturated with water, as eluant, to give four fractions containing neutral sugars, and elution with water gave a mixture of acidic substances (as barium salts) (E) which was examined later.

Fraction A. The syrup (0·90 g.) had $[\alpha]_D^{15}$ +111° (*c*, 0·96 in H_2O) and R_G 0·88 in solvent *B* (Found : OMe, 52·0. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%), and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 190°, $[\alpha]_D^{16}$ −63°→+34° (equil.) (*c*, 0·83 in Me_2CO).

Fraction B. The syrup (0·84 g.) had $[\alpha]_D^{15}$ +86° (*c*, 1·07 in H_2O) and R_G 0·71 (Found : OMe, 42·4. Calc. for $C_9H_{18}O_6$: OMe, 41·9%), and was identified as 2 : 3 : 6-tri-*O*-methyl-D-galactose by conversion into 2 : 3 : 6-tri-*O*-methyl-D-galactono-1 : 4-lactone, m. p. and mixed m. p. 98°, $[\alpha]_D^{17}$ −40°→−29° (equil.) (*c*, 0·55 in H_2O). As the 2 : 3 : 6- and the 2 : 4 : 6-trimethyl ether of D-galactose travel at similar rates on the chromatogram evidence for the presence of the 2 : 4 : 6-isomer in this fraction was sought but none was found : all attempts to prepare the aniline derivative failed and chromatographic examination of the tri-*O*-methylgalactonolactone indicated the presence of only the one component.

Fraction C. The sugar (0·28 g.) had $[\alpha]_D^{16}$ +37° (equil.) (*c*, 0·95 in H_2O), $[\alpha]_D^{15}$ +11° (equil.) (*c*, 1·93 in EtOH), and R_G 0·55 (Found : OMe, 18·5. Calc. for $C_7H_{14}O_5$: OMe, 17·4%), and crystallised [3] on removal of solvent, m. p. 115°. The methyl glycoside of the sugar was unattacked by periodate and the derived 3-*O*-methyl-L-rhamnono-1 : 4-lactone had $[\alpha]_D^{16}$ −20°→−18° (equil.) (*c*, 1·6 in H_2O).

Fraction D. The syrup (23 mg.) contained two sugars with R_G 0·55 and 0·45 and was examined after combination with a later fraction.

Examination of the Acidic Fraction.—The mixture of barium salts (E) was converted into the corresponding mixture of acids by removal of barium ions with Amberlite resin IR-120(H). The mixture was separated into two fractions by partition on cellulose, with, as eluant, butan-1-ol 50% saturated with water to which glacial acetic acid (5%, v/v) was added. Fraction 1 (0·70 g.) contained a main component having R_G 0·81 in solvent *C* together with a trace of a substance with R_G 0·95, and was unchanged by hot 2N-sulphuric acid. Fraction 2 (3·1 g.) contained substances with R_G less than 0·81 and was further hydrolysed by 2N-sulphuric acid at 100° for 24 hr. and the hydrolysate was neutralised with barium carbonate. The neutral sugars were separated from the barium salts of the acidic components by partition on cellulose, with light petroleum (b. p. 100—120°)–butan-1-ol, saturated with water, butan-1-ol saturated with water, and water as eluants. Fraction B(i) (45 mg.) had R_G 0·71 (solvent *B*) and fraction C(i) (110 mg.) had R_G 0·55. Fraction D(i) (33 mg.) contained sugars of R_G 0·55 and 0·45 and was combined with fraction D (total 56 mg.), $[\alpha]_D^{15}$ +57° (*c*, 0·63 in H_2O) [Found : OMe, 25·2. A mixture of di-*O*-methyl-hexose and mono-*O*-methylrhamnose (2 : 1) requires OMe, 25·7%]. Fraction F (20 mg.) travelled on the chromatogram at the same rate as rhamnose (R_G 0·30) and fraction G (20 mg.) had R_G 0·25 but was not examined further. Fraction 3, obtained as barium salts by

elution of the column with water, was converted into a mixture of acids by de-ionisation with Amberlite resin IR-120(H), and when taken to dryness gave a syrup (1·2 g.) in which one main component (R_G 0·45 in solvent *C*) was present, together with traces of other compounds.

Reduction of the Acidic Fractions with Lithium Aluminium Hydride.—The two acidic fractions 1 and 2 were combined (2·1 g.) and refluxed with methanolic 2% hydrogen chloride for 10 hr. After neutralisation with silver carbonate, the solution was taken to dryness, the resulting syrup was dissolved in dry tetrahydrofuran (10 c.c.), and the solution was added slowly to lithium aluminium hydride (1·0 g.) in tetrahydrofuran (10 c.c.). After 4 hr., the excess of hydride was destroyed by water, the solution was acidified with dilute sulphuric acid, the sulphate and aluminium ions were removed as precipitates on addition of barium hydroxide, and the barium and lithium ions were removed as the insoluble carbonates. The resulting solution was taken to dryness, to yield a syrup (1·1 g.), which was hydrolysed by N-sulphuric acid at 100° for 16 hr., and the hydrolysate was separated on cellulose, eluants being light petroleum (b. p. 100—120°)–butan-1-ol, saturated with water, and butan-1-ol 50% saturated with water, to give four fractions.

Fraction a. The syrup (0·111 g.) had $[\alpha]_D^{16}$ +73° (*c*, 0·63 in H_2O) and R_G 0·85 in solvent *B* (Found : OMe, 40·3. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Methylation of the sugar and hydrolysis of the resulting methyl glycoside yielded 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, m. p. 82° and $[\alpha]_D^{14}$ +81° (equil.) (*c*, 0·32 in H_2O), and the sugar was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. 146°.

Fraction b. The sugar (0·068 g.), $[\alpha]_D^{16}$ +85° (*c*, 0·99 in H_2O) and R_G 0·71 (Found : OMe, 40·5. Calc. for $C_9H_{18}O_6$: OMe, 41·9%), was identified as 2 : 3 : 6-tri-*O*-methyl-D-galactose by conversion into 2 : 3 : 6-tri-*O*-methyl-D-galactono-1 : 4-lactone, m. p. and mixed m. p. 97°.

Fraction c. The sugar (0·154 g.), $[\alpha]_D^{15}$ +14° (*c*, 0·57 in EtOH) and R_G 0·55, crystallised and had m. p. and mixed m. p. (with 3-*O*-methyl-L-rhamnose) 115° (Found : OMe, 17·0. Calc. for $C_7H_{14}O_5$: OMe, 17·4%).

Fraction d. The syrup (0·25 g.) had $[\alpha]_D^{15}$ +103° (*c*, 0·84 in H_2O) and R_G 0·46 in solvent *B*, and was identified as 2 : 3-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 154°, $[\alpha]_D^{19}$ −46·5°→+12° (equil.) (*c*, 1·0 in EtOH).[17]

Reduction of the Methylated Gum.—The methylated gum (0·50 g.) was dissolved in dry tetrahydrofuran (10 c.c.) and added slowly to lithium aluminium hydride (0·25 g.) in tetrahydrofuran (10 c.c.). After 2 hr. at room temperature, the solution was refluxed for 2 hr. and set aside overnight. Excess of hydride was destroyed by ethyl acetate, and the solution was acidified with dilute sulphuric acid and extracted with chloroform. The chloroform extract yielded the reduced methylated gum (A) (0·39 g.), $[\alpha]_D^{18}$ +46° (*c*, 1·44 in $CHCl_3$) (Found : OMe, 31·6%).

The reduced methylated gum A (0·30 g.) was further methylated by four treatments with methyl iodide and silver oxide, to give reduced methylated gum (B) (0·26 g.), $[\alpha]_D^{17}$ +34° (*c*, 1·12 in $CHCl_3$) (Found : OMe, 40·8%).

Hydrolysis of the Reduced Methylated Gums.—The reduced methylated gums (*ca.* 100 mg.) were hydrolysed by formic acid at 100° for 4 hr. and after removal of formic acid with N-hydrochloric acid at 100° for 16 hr. After neutralisation with silver carbonate the hydrolysates were separated on extractive-free Whatman 3MM paper with solvent *B*. The appropriate sections of the papers were extracted with hot methanol, and the extracts were filtered and taken to dryness. The annexed weights of sugars are corrected for blanks.

Sugar	R_G in solvent *B*	Wt. of sugar from gum A (89·1 mg.)	Wt. of sugar from gum B (86·9 mg.)
Tetra-*O*-methylglucose	1·00	—	5·1
Tetra-*O*-methylgalactose	0·88	15·9	12·7
Tri-*O*-methylglucose	0·85	15·9	—
Tri-*O*-methylgalactose	0·71	16·4	28·4
Mono-*O*-methylrhamnose	0·55	17·2	15·4
Di-*O*-methylgalactose	0·46	20·8	3·6
Rhamnose	0·30	1·2	0·8

Purification and Properties of Khaya senegalensis *Gum.*—The gum was obtained as colourless to dark brown nodules admixed with small particles of bark. One particular nodule had OMe, 2·2, and OAc, 2·7%, and chromatographic examination of the hydrolysate showed galactose, arabinose, rhamnose, and a complex mixture of acidic substances. The gum was purified by dissolving it in 4% sodium hydroxide solution, removing the mechanical impurities at the centrifuge, acidifying the resulting solution with hydrochloric acid, and precipitating the polysaccharide with ethanol. After three reprecipitations from aqueous solution with ethanol, the gum was

isolated as a white powder, $[\alpha]_D^{16}$ +124° (*c*, 0·97 in H_2O) [Found : equiv., 412 (by titration); sulphated ash, 2·2; OMe, 1·2%].

Partial Hydrolysis of K. senegalensis *Gum.*—The purified gum (2·30 g.) was heated with N-sulphuric acid at 100° for 6 hr., the hydrolysate was neutralised with barium hydroxide, the excess of alkali was rapidly destroyed with carbon dioxide, and the filtrate was taken to dryness. The residue was fractionated on cellulose, eluant being butan-1-ol saturated with water plus 5% ethanol, to give four fractions. Fraction 1 (0·091 g.) had $[\alpha]_D^{18}$ +10° (equil.) (*c*, 1·02 in H_2O) and after recrystallisation from moist butan-1-ol had m. p. and mixed m. p. (with L-rhamnose hydrate) 92°. Fraction 2 (0·048 g.) had $[\alpha]_D^{17}$ +113° (equil.) (*c*, 0·79 in H_2O) and after recrystallisation from ethanol had m. p. and mixed m. p. (with L-arabinose) 156°. Fraction 3 (0·370 g.) had $[\alpha]_D^{18}$ +80° (equil.) (*c*, 2·14 in H_2O) and after recrystallisation from methanol had m. p. and mixed m. p. (with D-galactose) 164°. Fraction 4 was obtained by elution of the cellulose with water and consisted of the barium salts of acidic substances. The barium ions were removed with Amberlite resin IR-120, and the acidic fraction was freeze-dried to give a yellow powder (0·87 g.). Chromatographic examination in solvent *D* indicated a complex mixture of acids, R_{Gal} 0·80 (intense), 0·40, 0·19, and 0·09, similar to those formed on partial hydrolysis of *K. grandifolia* gum. The fraction had equiv. 348 (by titration) and hydrolysis with 2N-sulphuric acid gave rhamnose and galactose. A portion of the fraction was converted into the methyl ester methyl glycoside by heating it with methanolic hydrogen chloride, then reduced with potassium borohydride, and the product was hydrolysed and shown by chromatography to contain rhamnose, 4-*O*-methylglucose, galactose, and a trace of glucose.

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221. *The Constitution of an Oat-straw Xylan.*

By G. O. Aspinall and K. C. B. Wilkie.

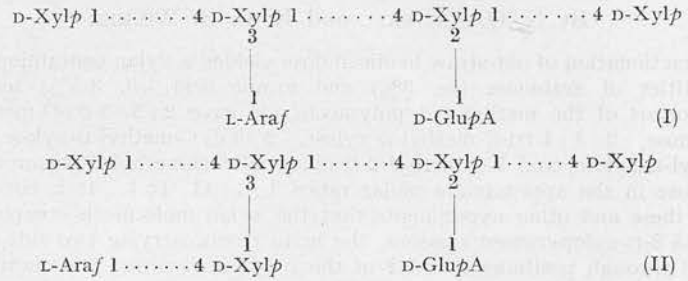
Fractionation of oat-straw hemicellulose yielded a xylan containing small quantities of arabinose (*ca.* 3%) and uronic acid (*ca.* 3·5%) residues. Hydrolysis of the methylated polysaccharide gave 2 : 3 : 5-tri-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, and 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose in the approximate molar ratios 1 : 1 : 41 : 1 : 1. It is concluded from these and other experiments that the xylan molecule is composed of 40—45 β-D-xylopyranose residues, the main chain carrying two side-chains linked through positions 3 and 2 of the D-xylose residues, and terminated by L-arabofuranose and 4-*O*-methyl-D-glucopyranuronic acid residues respectively. Possible structures for the polysaccharide are discussed.

In continuation of our structural studies of the hemicelluloses of lignified tissues, this paper describes the results of an examination of a xylan from oat straw. The hemicellulose was extracted from the delignified straw with cold aqueous sodium hydroxide, and hydrolysis of the polysaccharide indicated the presence of arabinose (5·6%) in addition to xylose residues. Repeated fractionations of the hemicellulose *via* the copper complex gave a xylan in which the proportion of arabinose residues had been reduced, but no xylan devoid of arabinose residues could be isolated. The xylan, thus obtained, gave on hydrolysis xylose and arabinose in the ratio 32 : 1 and had a uronic anhydride content of 3·5%. The acidic fraction obtained on hydrolysis of the xylan was converted into the methyl ester methyl glycoside, reduction of which with lithium aluminium hydride followed by hydrolysis of the reduction product yielded xylose and 4-*O*-methylglucose together with a trace of glucose. The acidic residues present in the polysaccharide, therefore, were those of glucuronic acid present for the most part as the 4-methyl ether.

The xylan was converted into the methylated derivative and the sugars obtained on hydrolysis of the methylated polysaccharide were partitioned on cellulose. The following sugars, which were isolated and characterised by the formation of crystalline derivatives—2 : 3 : 5-tri-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3-di-*O*-methyl-D-xylose and 2-*O*-methyl-D-xylose—were present in the molar ratios 0·8 : 1·0 : 41 : 1·2. In addition a tetra-*O*-methylaldobiouronic acid was isolated and shown to be 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose as reduction of its methyl ester methyl glycoside with lithium aluminium hydride followed by hydrolysis gave 2 : 3 : 4-tri-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose.

The main structural features of this oat-straw xylan are clear from these results. The isolation of the above aldobiouronic acid shows that each xylan chain contains a single D-glucuronic acid residue (probably as the 4-methyl ether) linked directly to the main chain through position 2 of a D-xylose residue. The isolation of 2 : 3 : 5-tri-*O*-methyl-L-arabinose accounts for all the arabinose residues present in the polysaccharide. The quantity of this compound isolated, together with that of 2-*O*-methyl-D-xylose, indicates that on the average each xylan carries a second side-chain terminated by an L-arabofuranose unit. It is not possible on the present evidence to distinguish between structures in which the L-arabofuranose residue is linked directly to the main chain (I) and those in which a side-chain of D-xylose residues is terminated by an arabinose residue (II), nor is it possible to indicate the relative positions in the main chain of the two branch points. The quantity of monomethylxylose isolated was only slightly in excess of that required by the branching point to the L-arabofuranose residue. The sugar, therefore, was not present in sufficient amount to accommodate a side-chain terminated by a D-xylopyranose residue, and it is probable that the excess arose from undermethylation of the polysaccharide and/or demethylation during hydrolysis. The results of periodate oxidation of the xylan were consistent with the picture of a xylan of 40—45 residues with side-chains terminated by an

L-arabofuranose residue and a 4-*O*-methyl-D-glucuronic acid residue, in that 1·0 mole of periodate was consumed per pentose residue and 1 mole of formic acid was released per 15·7 residues, corresponding to a molecule with one non-reducing D-xylopyranose residue per chain of 47 residues.



These results indicate that this xylan is of a similar molecular size to the wheat-straw xylan [1] previously examined in these laboratories. The occurrence of side-chains terminated by L-arabofuranose residues and linked to the main chain through position 3 of the D-xylose residue is a structural feature also found in the hemicelluloses of esparto grass [2] and of wheat-straw.[3–5] On the other hand, the linking of 4-*O*-methyl-D-glucuronic acid residues to position 2 of the xylose residues recalls a linkage characteristic of the wood hemicelluloses [6–8] rather than that of wheat straw.[9] It is of interest that in this investigation it was not possible by the fractionation of oat-straw hemicellulose to isolate a xylan devoid of arabinose residues, although it is still possible that such xylans may be present in oat straw. This fact, however, confirms the view that the xylans devoid of arabinose residues isolated from esparto grass [10] and wheat straw [1] by similar methods were true xylans and not artefacts from which the relatively labile arabofuranose residues had been removed during their isolation.

It is probable that the xylan studied in this investigation is only one of many xylans present in oat straw. In order, however, to decide whether the xylans from a particular source differ only in molecular size and in the number rather than in the nature of the residues linked as side-chains, or whether different structural features are present in these several xylans, it will be necessary for much more selective methods for the fractionation of such closely related polysaccharides to be developed.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the upper layers of the following solvents system (v/v): (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (B) butan-1-ol–ethanol–water (5 : 1 : 4); (C) butan-1-ol–acetic acid–water (4 : 1 : 5); and (D) benzene–ethanol–water (167 : 47 : 15).

Isolation and Fractionation of Oat-straw Hemicellulose.—Oat straw (variety Sun II, cut in September 1952; 365 g.) was delignified by Wise's method.[11] The holocellulose (*ca.* 220 g.) was extracted with sodium hydroxide solution (4%), the extract was acidified with glacial acetic acid, and the oat-straw hemicellulose (46 g.) was precipitated by the addition of an equal volume of acetone. The crude hemicellulose had $[\alpha]_D^{18}$ −94·7° (*c* 0·6 in N-sodium hydroxide) and chromatographic examination of the hydrolysate by Hirst and Jones's method [12] in solvent A showed the presence of xylose (94·4%) and arabinose (5·6%) (calc. as 100% pentose). The hemicellulose was fractionated by five successive precipitations of the copper complex formed on addition of Fehling's solution to a solution in sodium hydroxide (4%). The oat-straw xylan (19 g.) thus obtained had $[\alpha]_D^{19}$ −95·0° (*c* 0·5 in N-sodium hydroxide) [Found: ash (as sulphate), 0·8%; lignin, 3·3; uronic anhydride (by decarboxylation), 3·5; OMe, 0·5%] and was used in all subsequent investigations. Chromatographic examination of the hydrolysate showed the presence of xylose (97·0%) and arabinose (3·0%) (calc. as 100% pentose).

Examination of the Acidic Fraction from Xylan Hydrolysis.—Xylan (14 g.) was hydrolysed with 0·5N-sulphuric acid (100 c.c.) for 6 hr. at 100° and the hydrolysate was neutralised by passage through a column of Amberlite resin IR-4B. The resin was washed with 2N-sulphuric acid, the washings were neutralised with barium carbonate, and the filtrate was deionised with

Amberlite resin IR-120 and examined on the chromatogram. As large quantities of xylose were present the acidic sugars were re-adsorbed on Amberlite resin IR-4B, the neutral sugars were removed by elution with water, and the acidic sugars were isolated by the procedure previously described and freeze-dried, to give a solid (200 mg.). A portion (25 mg.) of the solid was converted into the methyl ester methyl glycoside, which was reduced with lithium aluminium hydride. The reduction product was hydrolysed with N-sulphuric acid and chromatographic examination of the hydrolysate showed the presence of 4-*O*-methylglucose, xylose, and glucose (trace).

Methylation of Oat-straw Xylan.—Xylan (17 g.) was methylated ten times with methyl sulphate and sodium hydroxide, and once with methyl iodide and silver oxide. The product (12·6 g.) was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 60—65°) to give a main fraction, soluble in boiling chloroform–light petroleum (30 : 70) (7·4 g.), which had $[\alpha]_D^{17}$ −87·0° (*c* 0·91 in $CHCl_3$) and was used in subsequent experiments (Found : OMe, 38·2%).

Hydrolysis of Methylated Xylan and Separation of Methylated Sugars.—The methylated xylan (5·49 g.) was hydrolysed successively with boiling methanolic 1% hydrogen chloride (600 c.c.) for 30 hr. and with 0·5N-hydrochloric acid (300 c.c.) at 100° for 15 hr. (constant rotation). Evaporation after neutralisation with silver carbonate yielded a syrup (5·99 g.). The syrup (5·93 g.) was fractionated on cellulose (88 × 4 cm.) [13] with light petroleum (b. p. 100—120°)–butan-1-ol (70 : 30), saturated with water, as eluant to give four fractions.

Fraction 1. Hypoiodite oxidation indicated 95·3% aldopentose, but chromatographic examination in solvent D showed the presence of 2 : 3 : 5-tri-*O*-methylarabinose, 2 : 3 : 4-tri-*O*-methylxylose, and 2 : 3-di-*O*-methylxylose. Although complete separation of the three sugars on filter sheets (Whatman 3MM) with solvent D was not always possible, in one case the three components were completely separated and hypoiodite oxidation showed tri-*O*-methylarabinose and tri- and di-*O*-methylxyloses to be present in the ratios 0·78 : 1·0 : 0·47, corresponding to 96, 124, and 58 mg. respectively of each sugar present in the fraction. Separation of the major part of fraction 1 gave chromatographically pure samples of the two tri-*O*-methylpentoses [fractions 1*a* (36 mg.) and 1*b* (42 mg.)] together with two fractions, containing respectively a mixture of the two tri-*O*-methylpentoses and di-*O*-methylxylose, which were not examined further. Fraction 1*a* had $[\alpha]_D^{19}$ +34·5° (*c* 0·6 in H_2O) and was identified as 2 : 3 : 5-tri-*O*-methyl-L-arabinose by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 136—137°, $[\alpha]_D^{20}$ −16·0° (*c* 1·0 in H_2O), which gave an identical *X*-ray powder photograph (by courtesy of Dr. C. A. Beevers) with that of the authentic amide. Fraction 1*b* crystallised on nucleation with 2 : 3 : 4-tri-*O*-methyl-D-xylose. After recrystallisation from dry ether the sugar had m. p. and mixed m. p. 90—91° and $[\alpha]_D^{18}$ +20° (equil.) (*c* 0·75 in H_2O), and gave an identical *X*-ray powder photograph with that of an authentic specimen. The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 95° and $[\alpha]_D^{18}$ +40° (*c* 0·1 in EtOH).

Fraction 2. The chromatographically pure syrup (4·91 g.) was shown by hypoiodite oxidation to be 94% aldopentose (Found : OMe, 34·4. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). Portions of the syrup were seeded with α- and β-forms of 2 : 3-di-*O*-methyl-D-xylose but in both cases the syrup crystallised as 2 : 3-di-*O*-methyl-β-D-xylose.[14] The two crystalline samples had m. p. 78—81°, $[\alpha]_D^{18}$ −20·3° (13 min.) ⟶ +27·8° (240 min., constant) (*c* 0·64 in H_2O), and m. p. 82—84° and $[\alpha]_D^{18}$ −18·1° (12 min.) ⟶ +26·2° (200 min., constant) (*c* 0·7 in H_2O) respectively, and had mixed m. p. 78·5—83·5°. The identity of the sugar was confirmed by conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 144—145°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 136·5°.

Fraction 3. The syrup (132 mg.) crystallised and had m. p. and mixed m. p. (with 2-*O*-methyl-D-xylose) 132—133° and $[\alpha]_D^{18}$ +34·0° (equil.) (*c* 1·0 in H_2O) (Found : OMe, 18·3. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). Hypoiodite oxidation indicated 93% aldopentose and paper ionophoresis [15] showed that no 3-*O*-methyl-D-xylose was present. The *X*-ray powder photograph was identical with that of an authentic sample and the derived 2-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 123·5°.

Fraction 4. The syrup (338 mg.), obtained by elution of the cellulose with water, was incompletely soluble in methanol. Purification was effected by dissolution in hot methanol, the insoluble residue was discarded, and the solution was decolorised with charcoal, to give a syrup (293 mg.), $[\alpha]_D^{19}$ +54° (*c* 0·5 in H_2O), R_G 0·12 in solvent C. A portion of the purified syrup (136 mg.) was refluxed for 6 hr. with methanolic 1·5% hydrogen chloride (50 c.c.), neutralised with silver carbonate, and taken to dryness. The resulting syrup was dissolved in dry ether

(25 c.c.), and the ethereal solution was added during 3 hr. to a boiling solution of lithium aluminium hydride (250 mg.) in ether (25 c.c.). After a further 2 hr. excess of hydride was destroyed by the addition of water, the solution was acidified with 2N-sulphuric acid and extracted with chloroform, and the chloroform extract was taken to dryness. The resulting syrup (78 mg.) was hydrolysed with 0·5N-hydrochloric acid (30 c.c.) for 8 hr. at 100°, neutralised with silver carbonate, and taken to dryness, to give a syrup (60 mg.). Chromatographic examination of the syrup showed sugars travelling at the same rates as 2 : 3 : 4-tri-*O*-methyl-D-glucose and 3(and/or 2)-methyl-D-xylose, but paper ionophoresis showed that only the 3-methyl ether was present. The major portion of the syrup was fractionated on filter sheets with solvent D, to give fractions *a* (21 mg.) and *b* (28 mg.). Fraction *a* was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the methyl β-D-pyranoside, m. p. and mixed m. p. 89·5°. Fraction *b* was identified as 3-*O*-methyl-D-xylose by conversion into the aniline derivative, m. p. 136—137°.

Periodate Oxidation of Oat-straw Xylan.—Oxidation of xylan (50 mg. batches) with potassium periodate solution by Halsall, Hirst, and Jones's method [16] gave the following results (expressed as moles of formic acid × 10^2 released per $C_5H_8O_4$ residue) : 7·25 (73 hr.); 8·76 (121 hr.); 9·31 (168 hr.); 11·01 (244 hr.); 12·27 (312 hr.); 15·77 (455 hr.). As the formic acid released did not reach a constant value, extrapolation to zero time gave a value corresponding to formic acid released from α-glycol scission, namely, 1 mole per 15·7 $C_5H_8O_4$ residues. The release of two mols. of formic acid from the reducing end-group and one mol. from the non-reducing end-group being assumed, this value corresponded to a chain length of 47 residues.

Oxidation of xylan with sodium metaperiodate solution showed that the polysaccharide consumed 1·0 mole of periodate (constant after 334 hr.) per $C_5H_8O_4$ residue. Hydrolysis of the periodate-oxidised polysaccharide showed the presence of a small quantity of xylose.

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ORGANIC CHEMISTRY

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ORGANIC CHEMISTRY.

1. INTRODUCTION.

By using a " plastic " model for initial and transition states, the contribution of steric strain to the energy of activation and the entropy of activation of nucleophilic bimolecular halogen exchange in alkyl halides has been calculated from first principles.

Phenanthrophenanthrene has been resolved and becomes the first known optically active hydrocarbon which owes its asymmetry to molecular overcrowding.

Impressive progress has been made in the identification of naturally occurring amino-acids and in the study of benzotropylium and allied cations, azulene synthesis, and the structure of the extraordinary growth factor mycobactin.

The common stereochemical pattern of many naturally occurring sesqui- and di-terpenes is being exposed from year to year by a combination of direct correlation with substances of known configuration and use of optical rotations. A valuable theory to explain the stereochemistry of steroids and triterpenes has been published; this theory is probably applicable to the smaller terpenes.

The structure of α-amyrin has been the subject of much discussion and experiment. Evidence has been described suggestive of the presence in the α-amyrins of a five-membered ring (E), with an attached *iso*propyl group. However, more evidence in favour of the older structure has also appeared. The position is still fluid, but the Reviewer is of the opinion that on balance the evidence favours the older structure.

In the steroid field one of the principle achievements has been the total synthesis of aldosterone. Important progress has also been made in the stereochemistry of the trimethyl-steroids.

Two outstanding events of the year were the deduction of the detailed structure of vitamin B_{12}, an excellent example of the use of chemical and *X*-ray techniques, and a brilliant two-stage synthesis of usnic acid from a simple derivative of acetophenone. The latter has cast light from a new angle on the oxidation of phenols.

G. B.
W. C.

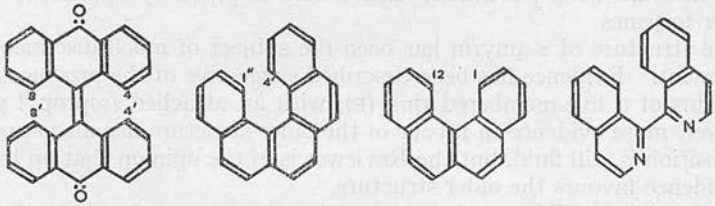
2. THEORETICAL.

Sterically Hindered Uniplanarity.—In numerous unsaturated compounds, changes in configuration, usually out-of-plane displacements, which decrease the intramolecular compression (destabilising) energy between non-bonded atoms also decrease the resonance (stabilising) energy. The preferred configurations, *i.e.*, those of lowest energy content, are those in which the rates of change of compression and resonance energy, with change of configuration, are equal. In these preferred configurations the molecules have energy in excess of what they would have had in the absence of steric interaction;

the excess comprises loss of resonance energy and gain of compression energy and is referred to as the steric strain.

It is generally found that non-bonded carbon atoms do not approach closer to each other than about 3·0 Å, and that the shortest distance between hydrogen atoms in aliphatic hydrocarbon crystals is about 2·5 Å. In dianthronylidene (1) the centre-to-centre distance between the overcrowded carbon atoms 4 and 4′, and 8 and 8′, is 2·90 Å; it is achieved by a 40° rotation of the benzene rings out of the plane of the central ethylenic system. The observed bond lengths and angles indicate that resonance interaction is considerably reduced, the molecule consisting of an isolated ethylenic group attached by single bonds to normal benzene rings.[1]

The overcrowding in 3 : 4-5 : 6-dibenzophenanthrene (2) is partially relieved by out-of-plane buckling of the molecule, which decreases the compression energy at the expense of the resonance energy. This buckling displaces the various carbon and hydrogen atoms in a direction normal to the original undisturbed molecular plane and is distributed over the five fused rings in such a manner as to cause the minimum distortion in any individual ring. It provides[2] a clearance of about 3·0 Å between the non-bonded carbon atoms 4′ and 1″. The loss of resonance energy caused by the deformation[3] is about 18 kcal. mole^{-1}, while the total steric strain energy may be about 28 kcal. mole^{-1}.



(1) (2) (3) (4)

These deformations affect chemical properties. It appears that for aromatic compounds, increase in departure from a uniplanar structure increases the localisation of the electrons which are usually delocalised in aromatic rings and hence leads, for example, to increased availability of electrons for reaction with free radicals. Methyl radicals add to aromatic compounds ($A + CH_3 \longrightarrow A{\cdot}CH_3$), and the relative rate constants are referred to as methyl affinities. Those of benzene, naphthalene, anthracene and naphthacene are 1, 22, 820, and 9250 respectively;[4] their logarithms vary linearly with the singlet–triplet excitation energies.[5] Now, whereas the methyl affinities of 2-, 3-, 4-, 5-, and 6-methylbenzo[*c*]phenanthrene are roughly the same and about equal to that of the parent hydrocarbon (3), overcrowding causes the methyl affinity to rise somewhat in the 1-methyl and considerably in the 1 : 12-dimethyl derivative.[6] Again, the logarithms

[1] E. Harnik and G. M. J. Schmidt, *J.*, 1954, 3295.
[2] A. O. McIntosh, J. M. Robertson, and V. Vand, *J.*, 954, 1661.
[3] C. A. Coulson and S. Senent, *J.*, 1955, 1813, 1819.
[4] M. Levy and M. Szwarc, *J. Chem. Phys.*, 1954, **22**, 1621; *J. Amer. Chem. Soc.*, 1955, **77**, 1949.
[5] M. Szwarc, *J. Chem. Phys.*, 1955, **23**, 204.
[6] M. Levy, M. S. Newman, and M. Szwarc, *J. Amer. Chem. Soc.*, 1955, **77**, 4225.

of the reaction constants for interaction of 1-chloro-2 : 4-dinitrobenzene and amino-naphthalenes, -anthracenes, and -phenanthrenes in ethanol are inversely proportional to the localisation energy as calculated for the hydrocarbon analogues of the amines. 4-Aminophenanthrene is an exception; this misfit is caused by the steric strain in this amine.[7]

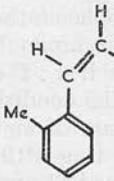
Strain similar to that in (2) would arise if 1 : 1′-di*iso*quinoline (4) participated in the ferroin reaction; the essential condition for this reaction is the specific grouping ·N:C·C:N·, forming part of an aromatic system and capable of forming a five-membered chelate ring with ferrous ion. In fact, the base (4) does not give the reaction [8] whereas 1-2′-pyridyl*iso*quinoline does.[9]

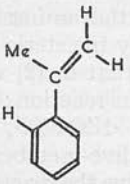
Spectroscopic evidence for the steric inhibition of uniplanarity with consequent reduction of conjugation has recently received further attention with a view to a quantitative interpretation. There are two different types of spectral manifestation: (i) decrease in absorption intensity (transition probability) without appreciable increase in the frequency (transition energy); and (ii) change in both. *o*-Substituted acetophenones, benzophenones,[10] and styrenes exhibit steric effects of type (i), *o*-substituted diphenyls those of type (ii), and 5-*o*-substituted 2 : 4-diaminophenylpyrimidines those of both types.[11] It is reasonable to assume that those of type (ii) are associated with transitions between the non-planar ground state and the non-planar excited state, whereas the former are associated with transitions between the non-planar ground state and the planar or near-planar excited state.[12] This assumption leads to the conclusion that in manifestations of type (i) the absorption process is mainly concerned with those molecules in which, during vibration, the interplanar angle $\theta \simeq 0°$; for in accordance with the Franck–Condon principle the relative positions of the atomic nuclei, including the value of θ, do not change during the process of absorption. The absorption intensity (ε) depends on the population distribution and the transition probability as functions of θ, and if we assume that the transition probability for $\theta = 0°$ is not affected appreciably by steric strain, the decrease in the absorption (as measured by $\varepsilon/\varepsilon_0 = r$) may be taken as a measure of the fraction of the molecules which, at any instant, have $\theta \simeq 0°$. Thus r may be a measure of the energy required to impose uniplanarity. Calculation has, however, taken another course: on the assumption that r is related to θ_1, the interplanar angle for the molecule in its lowest energy state, by $r = \cos^2 \theta_1$ (a relation which may be difficult to justify), r has been used in calculations of interplanar angles.[13, 14]

One condition for the display of steric effects of type (i) is that steric hindrance to planarity should not exceed about 3 kcal. mole^{-1}. It is pleasing to find that when the hindrance is expected to be about the same in different compounds, *i.e.*, when changes of resonance and compression energy severally with change of θ are similar in different compounds, the absorption intensities of these compounds afford similar values of r. For example, the absorption

[7] F. L. J Sixma, *Rec. Trav. chim.*, 1955, **74**, 168.
[8] F. H. Case, *J. Org. Chem.*, 1952, **17**, 471.
[9] H. Irving and A. Hampton, *J.*, 1955, 430.
[10] R. F. Rekker and W. T. Nanta, *Rec. Trav. chim.*, 1954, **73**, 969.
[11] P. B. Russell, *J.*, 1954, 2951.
[12] E. A. Braude, F. Sondheimer, and W. Forbes, *Nature*, 1954, **173**, 117.
[13] E. A. Braude and F. Sondheimer, *J.*, 1955, 3754.
[14] L. H. Klemm, H. Ziffer, J. W. Sprague, and W. Hades, *J. Org. Chem.*, 1955, **20**, 190.

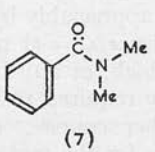
intensity of styrene is decreased by an *o*-methyl substituent [15] ($\tau = 0{\cdot}78$) to the same extent as by an α-methyl substituent ($\tau = 0{\cdot}78$) [cf. (5) and (6)].

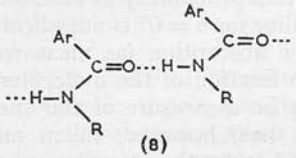

(5)


(6)

The absorption intensity of α-alkylstyrenes decreases progressively with increase in bulk of the alkyl substituent; [16] this indicates that steric inhibition of uniplanarity exists here. There is a small but significant hypsochromic effect. The absorption intensities of the compounds Ph·OR,[17] Ph·NHR, and Ph·COR decrease with increase in the bulk of the alkyl group (R) and are similarly interpreted.

Unlike benzamide and its *N*-methyl derivative, the *NN*-dimethyl derivative (7) cannot have a completely planar structure because of interference between one of the methyl groups and the benzene ring. This interference, which is apparent in the ultraviolet absorption of the compound, is probably relieved mainly by rotation about the Ph–CO bond since resonance interaction, and hence the need for uniplanarity, is greater about the Me_2N-CO bond.[18] The electric moments of *N*-monosubstituted benzamides indicate that the amide group is planar and that the less strained conformation has the substituent in the *cis*-position with respect to the carbonyl group. As a consequence,[19] these compounds seem to associate to form linear complexes (8) whereas the unsubstituted amides form ring complexes (9).

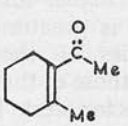

(7)

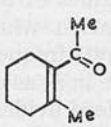

(8) (9)

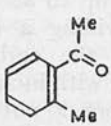
An interesting problem arises when attempting to relate steric hindrance of uniplanarity in *cyclo*hexenyl derivatives with that in the corresponding phenyl derivatives. It has been argued [20] that 1-acetyl-2-methyl*cyclo*hexene is more stable in the *s-trans*-conformation (10) than in the *s-cis*-conformation (11). There is, however, no reason for the benzenoid analogue to have a conformation other than one approximating to (12). Analysis [21] of the electronic moments of a number of αβ-unsaturated aldehydes and

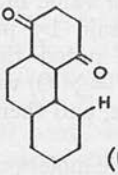
[15] P. Ramart-Lucas and J. Hoch, *Bull. Soc. chim. France*, 1935, 327; 1938, 848; E. A. Braude and F. Sondheimer, *J.*, 1955, 3773.
[16] P. Ramart-Lucas, Proc. XIth Inst. Congr. Pure Appl. Chem., London, 1947, Vol. II, p. 267; C. G. Overberger and D. Tanner, *J. Amer. Chem. Soc.*, 1955, **77**, 369.
[17] G. Baddeley, N. H. P. Smith, and M. A. Vickers, in the press.
[18] J. T. Edwards and S. C. R. Meacock, *Chem. and Ind.*, 1955, 536.
[19] J. E. Worsham, jun., and M. E. Hobbs, *J. Amer. Chem. Soc.*, 1954, **76**, 206.
[20] E. A. Braude and C. J. Timmons, *J.*, 1955, 3766.
[21] G. K. Estok and J. S. Dehn, *J. Amer. Chem. Soc.*, 1955, **77**, 4769.

ketones indicates that the β-disubstituted unsaturated ketones have predominantly the *s-cis*-conformation, *e.g.*, see (11), that the aldehydes have the *s-trans*-, and that the β-monosubstituted unsaturated ketones have approximately equal contributions from both the *s-trans*- and the *s-cis*-conformation. The quasi-theoretical and experimentally established stability sequence of five stereoisomeric perhydro-1 : 4-dioxophenanthrenes (13) indicates [22] that the energy increment due to the non-bonded interaction of the oxygen atom of a carbonyl group and a β-situated C–H bond in an eclipsed (equatorial) position is 0·8—1·2 kcal. mole^{-1}. The energy of non-bonded interaction of carbonyl and *o*-methyl group in (11) and (12) will not be greater than this amount and may be appreciably less.


(10)


(11)


(12)


(13)

A qualitative correlation has been made [23] of $\lambda_{max.}$ values for αβ-unsaturated carbonyl compounds in which there is bond deformation in one part of the chromophore. The excitation energy is found to be lower for compounds in which the electron displacement of excitation is away from a site of strain. This relationship is in accordance with the idea [24] that greater electronegativity is associated with greater *s*-character of the carbon bonds and hence with less directional and more deformable bonds.

The absorption intensity for the *o*-halogenonitrobenzenes is in the order F > Cl > Br > I, which is the reverse of that for the absorpton intensity of the *p*-halogenonitrobenzenes and the 2-halogenopyridines. The difference is interpreted as being caused by steric inhibition of uniplanarity of nitro- and phenyl-groups by the *o*-halogen substituent; a similar effect is not possible in the 2-halogenopyridines.[25]

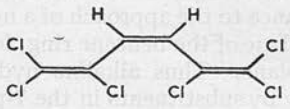
Polymethylene chains have conformational preferences which may oppose the uniplanarity required for maximum conjugation. Compounds of type (14) and (15), in which X = >CO, >C:N·NHAr, >C:N·OH, >C:N·NH·CO·NH_2,[15, 26, 27, 28] >NH, >NMe, >N·COR, >O;[17] XY = –CH:CH–,[26, 27] –NH·CO–;[29] and n = 5—9; and those of type (16), in which Y = >CO and >C:N·OH, and n = 13—18,[30] have been studied and their properties discussed. The data do not, however, provide a quantitatively consistent pattern. The spectroscopic results for compounds of type (16), where Y is a carbonyl group, show the interplanar angle of the carbonyl and

[22] P. A. Robins and J. Walker, *J.*, 1955, 1789; *Chem. and Ind.*, 1955, 727.
[23] W. M. Schubert and W. A. Sweeney, *J. Amer. Chem. Soc.*, 1955, **77**, 2297.
[24] A. D. Walsh, *Discuss. Faraday Soc.*, 1947, **2**, 18.
[25] H. C. Brown and D. H. McDaniel, *J. Amer. Chem. Soc.*, 1955, **77**, 3752; H. E. Ungnade, *ibid.*, 1954, **76**, 1601.
[26] G. Baddeley and J. Chadwick, *J.*, 1951, 368.
[27] R. Huisgen, W. Rapp, I. Ugi, H. Walz, and E. Mergenthaler, *Annalen*, 1954, **586**, 1.
[28] F. Ramirez and A. F. Kirby, *J. Amer. Chem. Soc.*, 1954, **76**, 1037.
[29] R. Huisgen, I. Ugi, H. Brade, and E. Rauenbusch, *Annalen*, 1954, **586**, 30.
[30] R. Huisgen, W. Rapp, I. Ugi, H. Walz, and I. Glogger, *ibid.*, p. 52.

when X is >CO ($r = 0{\cdot}82$) than when X is >O ($r = 0{\cdot}23$). This relation is also apparent in the optical stabilities of diphenyls and related compounds, *e.g.*, electron-withdrawing substituents Z in the amine (18) increase the conjugation of the nitrogen atom and the benzene ring and thereby increase the rate of racemisation. Electron-supplying substituents have the opposite effect.[36]

These considerations cast doubt on the significance of interplanar angles derived from scale models, and of calculations in which the conformational preference of polymethylene chains is assumed to prevail over that of conjugation.[13] Conventional steric considerations can be misleading, *e.g.*, systems containing *trans*-fused *cyclo*pentane rings are more readily formed than steric considerations would suggest[37] and optically active 1 : 1′-dinaphthyl derivatives racemise even when a mechanical interpretation by use of models indicates this to be impossible.[38] Further, there is need for caution in ascribing to steric hindrance of uniplanarity small differences of intensity of absorption between even closely related compounds :[39] *e.g.*, whereas the *cis*- and the *trans*-forms of penta-1 : 3-diene and pent-3-en-1-yne absorb with practically equal intensity, extension of the chromophore by addition of a methoxycarbonyl group in each case results in an appreciable difference between geometrical isomers, the ratio being remarkably constant. Steric inhibition of uniplanarity can be discounted here; apparently other factors are involved.[39] It should be noted that stereoisomeric hexa-2 : 4-dienoic esters differ appreciably in both $\lambda_{max.}$ and absorption intensity, the *trans-trans*-isomer absorbing maximally at the shortest wavelength, exactly the opposite behaviour to that predicted on the basis of one of Zechmeister's generalisations.[40] This rule must be inverted for simple, laterally unsubstituted polyenes.[39, 41]

Steric hindrance of uniplanarity in 1 : 1 : 2 : 5 : 6 : 6-hexachlorohexa-1 : *cis*-3 : 5-triene (19) and 3 : 4-dibromohexachlorohexa-1 : *trans*-3 : 5-triene (20) is clearly indicated by their absorption spectra.[42]



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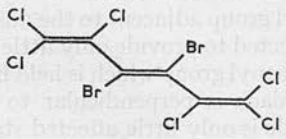


(20)

Intermolecular Strain.—Most chemical reactions involve one or more of a number of possible steric effects and, for any one reaction, it is seldom apparent which will be the most important one and whether it will outweigh electronic influences. A complete theory of the effect of substituents on the course and rate of reaction and on equilibrium will incorporate a quantitative treatment of spatial and electronic influences; in the meantime, evidence for

[36] R. Adams and K. V. Y. Sundstrom, *J. Amer. Chem. Soc.*, 1954, **76**, 5474.
[37] L. N. Owen and A. G. Peto, *Chem. and Ind.*, 1955, 65.
[38] F. Bell and W. H. D. Morgan, *J.*, 1954, 1716.
[39] J. L. H. Allan, E. R. H. Jones, and M. C. Whiting, *J.*, 1955, 1862.
[40] L. Zechmeister, *Experimentia*, 1954, **10**, 1.
[41] P. Nayler and M. C. Whiting, *J.*, 1954, 4006.
[42] A. Roedig and K. Kiepert, *Chem. Ber.*, 1955, **88**, 733.

the relative importance of the various influences and their dependence on reaction mechanism is rapidly accumulating.

This sub-section is concerned with recent examples of reactions in which non-bonding interaction of one reactant with another (intermolecular strain) increases the energy content of the transition state or product and thereby influences reaction rate or equilibrium. Other types of strain are discussed in later sub-sections.

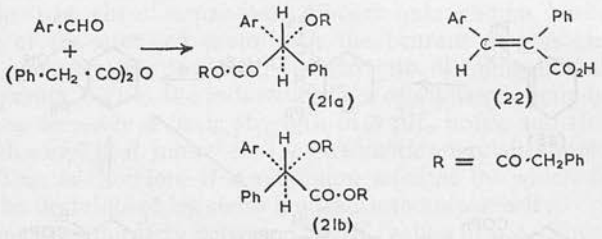
This intermolecular strain has a pronounced influence on the interaction of iodine monochloride with 1 : 3 : 5-tri-*tert.*-butyl- and pentaethyl-benzene [43] and on the methyl affinity of chloranil [44] and di-*tert.*-butyl-1 : 4-benzoquinone.[45] The structure of the product of the primary addition of the methyl radical is still undetermined and it is not clear whether the reacting radical attacks initially at an oxygen atom or at an ethylenic bond of the quinone. The latter is indicated by the steric effect : it seems that whenever the ethylenic bond is shielded by bulky atoms or groups the reactivity of the quinone decreases. The methyl affinities of styrene (792), *trans*-stilbene (105), triphenylethylene (46), and tetraphenylethylene (< 10) show that steric strain is more important than resonance stabilisation of the resulting radicals in determining these relative rates.[46] *o*-Methyl substituents have two mutually opposing steric effects on the dissociation of hexaphenylethane : by imposing a greater degree of non-planarity on the triphenylmethyl radical they lower its stability and thereby hinder dissociation, while facilitating this process by providing additional overcrowding in the ethane. The latter effect outweighs the former.[47]

Intermolecular strain may determine the course of reaction. Whereas the alkaline rearrangement of 3- and 4-chlorobenzil [48] and 2- and 3-chlorophenanthraquinone [49] results in the preferential migration of the substituted ring, that of 2-chlorobenzil and 1-chlorophenanthraquinone results in the preferential migration of the unsubstituted ring.[50] Apparently, steric hindrance by an adjacent chlorine atom overshadows electronic effects transmitted within the molecule, and the hydroxide ion attacks more readily at the carbonyl group adjacent to the unsubstituted ring. However, *o*-substituents are expected to provide only little hindrance to the approach of a nucleophile to a carbonyl group which is held in the plane of the benzene ring, for the line of approach is perpendicular to this plane. Thus alkaline hydrolysis of phthalide is only little affected sterically by substituents in the 7-position.[51]

The product of the condensation of aryl aldehydes with phenylacetic anhydride in the presence of sodium phenylacetate is mainly the *trans*-cinnamic acid (22); in this the bulkiest groups are *cis* with respect to one another. Apparently, therefore, intermolecular strain rather than the relative stability of the possible products is the determining factor in the

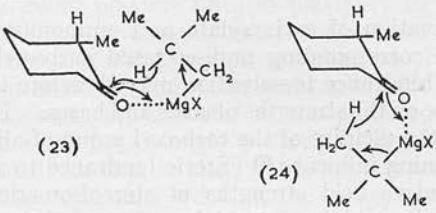
[43] N. Ogimachi, L. J. Andrews, and R. M. Keefer, *J. Amer. Chem. Soc.*, 1955, **77**, 2164, 4202.
[44] A. Rembaum and M. Szwarc, *ibid.*, p. 4468.
[45] F. J. L. Aparicio and W. A. Waters, *J.*, 1952, 4666.
[46] F. Leavitt, M. Levy, M. Szwarc, and V. Stannett, *J. Amer. Chem. Soc.*, 1955, **77**, 5493.
[47] W. Theilacker and M.-L. Wessel-Ewold, *Annalen*, 1955, **594**, 214.
[48] M. T. Clark, E. C. Hendley, and O. K. Neville, *J. Amer. Chem. Soc.*, 1955, **77**, 3280.
[49] D. G. Ott and G. G. Smith, *ibid.*, 1954, **76**, 2325.
[50] G. G. Smith and D. G. Ott, *ibid.*, 1955, **77**, 2342.
[51] J. Vène and J. Tirouflet, *Bull. Soc. chim. France*, 1954, 220.

stereochemical course of the reaction. The formation of the intermediate (21a) involves less steric compression than that of the alternative (21b) and affords the *trans*-cinnamic acid by elimination of the elements of phenylacetic acid.[52] Similarly, polymerisation of vinyl chloride and acetate seems to



proceed in such a manner that like groups are at maximum distance apart in the addition process.[53]

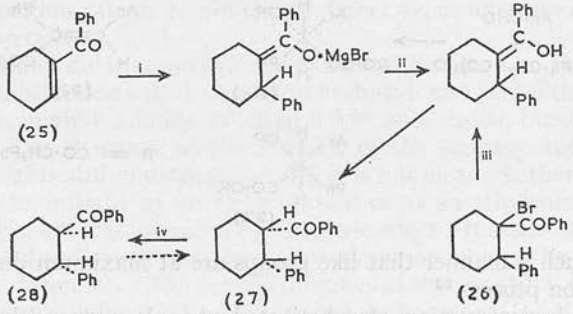
Catalytic hydrogenation of substituted phenols and *cyclo*hexanones at room temperature gives alcohols containing a high proportion of the less stable isomer with an axial hydroxyl group, *i.e.*, the *cis*-1 : 2-, *trans*-1 : 3-, and *cis*-1 : 4-isomers. It seems that, as in many previous catalytic hydrogenations, the initial addition is *cis* and occurs on the more accessible side of the double bond.[54] In accordance with this generalisation, the proportions of saturated steroids of the A/B-*cis*- and the A/B-*trans*-series formed by catalytic hydrogenation of 3α-substituted Δ^5-steroids show that the bulkier the 3α-substituent the larger is the proportion of the saturated A/B-*cis*-steroid.[55] Reduction of 2-methyl*cyclo*hexanone with *iso*butylmagnesium bromide (see 23) is probably directed by steric hindrance to the approach of the β-CH group of the Grignard reagent to the carbonyl-carbon atom; the *cis*-secondary alcohol is the main product. On the other hand, reduction



with *tert.*-butylmagnesium bromide (see 24) gives mainly the *trans*-alcohol—the more stable form. In this reaction the approach of the β-CH group to the carbonyl group is not effectively hindered and its direction is that which involves least hindrance to change in shape of the six-membered ring.[56]

[52] M. Crawford and G. W. Moore, *J.*, 1955, 3445; C. L. Arcus and D. G. Smyth, *J.*, 1955, 34.
[53] C. L. Arcus, *J.*, 1955, 2801.
[54] E. G. Peppiatt and R. J. Wicker, *Chem. and Ind.*, 1955, 747; R. Cornubert, G. Barraud, M. Cormier, M. Descharmes, and H. G. Eggert, *Bull. Soc. chim. France*, 1955, 400.
[55] J. R. Lewis and C. W. Shoppee, *J.*, 1955, 1365.
[56] P. Anziani, A. Aubry, G. Barraud, M. M. Clandon, and R. Cornubert, *Bull. Soc. chim. France*, 1955, 408.

It has been suggested [57] that the stereochemistry of the ketonisation of enols is determined by a preferential attack of a proton carrier on the less hindered side of the enol double bond. Thus the ketones (25) and (26) give the product (27), rather than (28), the thermodynamically more stable isomer.



Reagents: i, Ph·MgBr. ii, H^+. iii, HI. iv, NaOEt.

According to the free-energy relation between the basic strengths of pyridine bases on the one hand and the catalytic effect of these bases on the rate of coupling of *p*-chlorobenzenediazonium ions with 2-naphthol-6 : 8-disulphonic acid on the other, catalysis by 2-picoline and 2 : 6-lutidine is respectively 3 and 10 times smaller than is to be expected. Thus, in contrast to other protolytic reactions of pyridine derivatives,[58] reactions of base with the thermodynamically metastable intermediate (29) show that steric strain may be important for proton-transfer processes.[59]

$$Ar{\cdot}N_2^+ + Ar'H \rightleftharpoons [Ar{\cdot}N{:}N{\cdot}Ar'H]^+ \quad (29)$$

$$[Ar{\cdot}N{:}N{\cdot}Ar'H]^+ + Base \longrightarrow Ar{\cdot}N{:}N{\cdot}Ar' + H{\cdot}Base^+$$

Energies of solvation of carboxylate and ammonium ions are greater than those of the corresponding undissociated carboxylic acids and free amines, and steric hindrance to solvation may therefore have an important weakening influence on the strengths of acids and bases. Thus, accumulation of alkyl groups in the vicinity of the carboxyl group of aliphatic acids has a pronounced weakening influence.[60] Steric hindrance to solvation seems to determine the relative acid strengths of stereoisomeric monosubstituted *cyclo*hexanecarboxylic acids; equatorial groups are the more accessible and the isomer with the carboxyl and the other substituent equatorial is a stronger acid than its isomer with one group axial.[61] Similarly, amino- or dimethylamino-cholestane with an equatorial basic group is a stronger base than its epimer, and the more hindered the position on the cholestane nucleus, in the order 3- < 2- < 6-, the greater the difference between the epimers.[62] The

[57] H. E. Zimmerman, *J. Org. Chem.*, 1955, **20**, 549.
[58] H. C. Brown and R. R. Holmes, *J. Amer. Chem. Soc.*, 1955, **77**, 1727; H. C Brown and R. H. Horowitz, *ibid.*, p. 1733.
[59] H. Zollinger, *Helv. Chim. Acta*, 1955, **38**, 1597, 1617.
[60] G. S. Hammond and D. H. Hoyle, *J. Amer. Chem. Soc.*, 1955, **77**, 338.
[61] J. F. J. Dippy, S. C. R. Hughes, and J. W. Laxton, *J.*, 1954, 4102.
[62] C. W. Bird and R. C. Cookson, *Chem. and Ind.*, 1955, 1479.

importance of steric hindrance to uniplanarity in *enhancing* the strengths of aromatic acids and bases [63] has recently received additional confirmation,[64, 65] and these two contrasting steric factors, one weakening, the other strengthening, are sufficient to give a qualitative account of steric effects on the strengths of carboxylic acids and organic bases.

The basic strengths of benzo- and dibenzo-quinuclidine, bases in which conjugation of the nitrogen atom with the benzene ring is excluded on grounds of geometry, are less than the strength of quinuclidine by about 3 and 6 pK_a units. Thus the inductive effect of a phenyl group is shown to bring about a decrease of basic strength of 3 pK_a units, and this decrease leads to a value of 4 kcal. mole^{-1} for the mesomeric energy in simple aromatic amines.[65] This is therefore the maximum amount by which these compounds can be destabilised by steric hindrance to uniplanarity.

Deviations from linearity between the pK_a values of substituted pyridine bases, in which steric hindrance to uniplanarity does not occur, and those of the correspondingly substituted aromatic acids and bases have been used to provide a measure of the *ortho*-effect of halogen and alkyl substituents.[66]

The rates of reaction of alkyl halides with monoalkylpyridines decrease sharply from methyl to ethyl, and from ethyl to *iso*propyl halide; the energies of activation show a corresponding increase while the entropies of activation remain sensibly constant. The introduction of an alkyl group in the 2-position of pyridine effects a decrease in rate which is very pronounced in the case of 2-*tert.*-butylpyridine.[67] It is pleasing to find that steric strain has an influence on the activation energy of these reactions which is very similar to its influence on the energy content of the addition products of pyridine bases with Lewis acids.[67]

The contribution of steric strain to the energy of activation and the entropy of activation of nucleophilic bimolecular halogen exchange in alkyl halides [Finkelstein substitution, see (30)] has been calculated [68] from first principles. A " plastic " model is adopted for initial and transition states; in each, it permits every possible form of plasticity to the three reacting atoms but allows only conformational rotations, and not bond-stretching or bond-bending, within the alkyl groups attached to the α-carbon atom. Non-bonding pressures in the transition state move the halogen atoms off the line through C_α and perpendicular to the plane containing the three bonds to R, R′, and R″ by an angle θ which is large for substitution in *neo*pentyl halide, small for that in ethyl halide, and zero for that in methyl and *tert.*-butyl halide. For each reaction, the energy terms (i) to bend the C_α–X bond of the initial molecule through the angle θ and to stretch it to the length r, (ii) to force the entering halide ion against the α-C atom at the angle θ and to the separation r, and (iii) to do the necessary work against the pressures exerted on X in the (r, θ) configuration by the atoms or groups R, R′, and R″, are calculated for each r, θ. The totals are plotted as a

[63] G. Baddeley, *Nature*, 1939, **144**, 444.
[64] J. F. J. Dippy, S. C. R. Hughes, and J. W. Laxton, *J.*, 1954, 1470.
[65] B. M. Wepster, *Rec. Trav. chim.*, 1952, **71**, 1159, 1171; S. M. H. Van der Krogt and B. M. Wepster, *ibid.*, 1955, **74**, 161.
[66] D. H. McDaniel and H. C. Brown, *J. Amer. Chem. Soc.*, 1955, **77**, 3756.
[67] H. C. Brown and A. Cahn, *ibid.*, p. 1715.
[68] P. B. D. de la Mare, L. Fowden, E. D. Hughes, C. K. Ingold, and J. D. H. Mackie, *J.*, 1955, 3200.

surface representing potential energy as a function of the configuration of the reacting system; each surface has the form of a basin, the lowest point of which gives the configuration and energy of the transition state. The rotational entropies depend on the spatial co-ordinates of the minima, whilst the vibrational entropies depend on the curvature of the surfaces around the minima. Thus the height of the surface relative to that for halogen exchange in methyl halide determines the contribution of steric strain to the energy of activation, and their relative shapes its contribution to the entropy of activation. As shown in the Table, the calculated steric energies (ΔW_S) fall

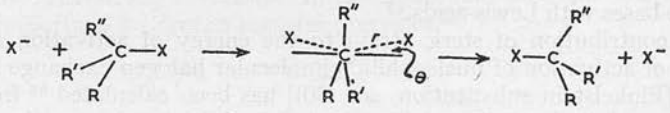
Comparison of calculated and observed effects of alkyl structure on energies of activation (kcal. mole^{-1}) for the reaction :

$$Br^- + \text{Alkyl-Br} \longrightarrow \text{Br-Alkyl} + Br^-.$$

Alkyl :	Me	Et	Pr^i	Bu^t	Pr	Bu^i	CH_2Bu^t
ΔW_S	0	0·8	1·6	2·5	0·8	2·3	7·3
ΔE_A	0	1·7	3·9	6·0	1·7	3·1	6·2
Difference	0	0·9	2·3	3·5	0·9	0·8	−1·1
n *	0	1	2	3	1	1	1

* The number of α-alkyl substituents.

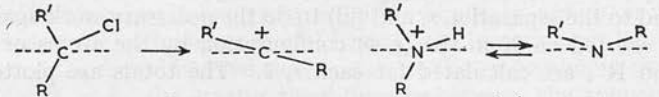
below the relevant differences of experimental activation energy (ΔE_A), and the authors interpret the discrepancies in terms of polar effects (ΔW_P) which cause each α-alkyl substituent to raise the energy of activation by about 1 kcal. mole^{-1}. Neglect of deformation in the α-alkyl substituents is considered to be the biggest single source of error in the calculations.



(30)

Intramolecular Steric Effects.—*Reaction rate and equilibrium.* Differences of intramolecular spatial configuration between (*a*) reactant and transition state and (*b*) reactant and product may effect differences of non-bonding interaction which will influence (i) the reaction rate and (ii) the position of the equilibrium. This influence is not readily disentangled from electronic effects and is therefore difficult to estimate.

Intramolecular steric hindrance to ionisation of aralkyl chlorides Ar·CRR'Cl has been recognised by relating [69] log k for their ionisation (31)



(31) (32)

to pK_a for dissociation (32) of the corresponding anilinium ions $^+$NHArRR'. The electronic displacements incurred by these two processes are oppositely directed and, steric effects apart, a change in constitution which increases

[69] G. Baddeley, J. Chadwick, and H. T. Taylor, *J.*, 1954, 2405.

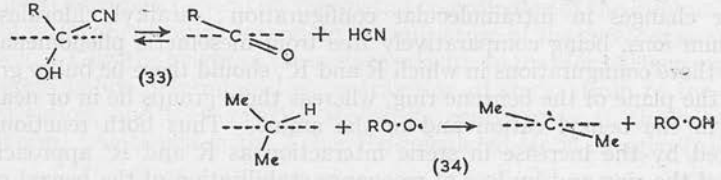
the rate of ionisation decreases the dissociation; the amines of greater basic strength are those which correspond to the more reactive chlorides and *vice versa*; $d \log k/d\ pK_a$ is positive. On the other hand, these reactions involve similar changes in intramolecular configuration: aralkyl chlorides and anilinium ions, being comparatively free from mesomeric phenomena, will prefer those configurations in which R and R', should these be bulky groups, avoid the plane of the benzene ring, whereas these groups lie in or near this plane in the benzyl cation and in the amine. Thus both reactions are hindered by the increase in steric interaction as R and R' approach the plane of the ring and by loss of resonance stabilisation of the benzyl cation and of the amine if these, for steric reasons, are not approximately planar. In a series of chlorides and amines in which the members differ mainly in the magnitude of this steric effect, the more basic amines will be those which correspond to the less reactive chlorides and *vice versa*; $d \log k/d\ pK_a$ will be negative.

Rates of solvolysis of the chlorides R·CHPhCl,[69] RR'CPhCl, Ph·$\overline{\text{CCl}\cdot[\text{CH}_2]_{n-1}}$, and *o*-$\overline{\text{C}_6\text{H}_4\cdot\text{CHCl}\cdot[\text{CH}_2]_{n-3}}$ [70] have been compared with the basic strengths of the corresponding amines R·NHPh, RR'NPh, Ph·$\overline{\text{N}\cdot[\text{CH}_2]_{n-1}}$, and *o*-$\overline{\text{C}_6\text{H}_4\cdot\text{NH}\cdot[\text{CH}_2]_{n-3}}$ respectively. In general, reactivity of the chlorides *decreases* and basic strength of the amines *increases* with increase in the bulk of R and R' and in the value of *n*. This relation and the fact that it does not obtain when the benzene ring is replaced by a saturated hydrocarbon moiety support the view that ionisation of the aralkyl chlorides and dissociation of the anilinium ions effect changes of conformation which increase steric interaction. The energies of activation for ionisation of the chlorides R·CHPhCl indicate that hindrance by R is equivalent to 1 kcal. mole^{-1} when R is a primary alkyl group, and to 3·5 and 6·0 kcal. mole^{-1} when R is a secondary or tertiary alkyl group respectively. Hindrance to ionisation of the chlorides *o*-$\overline{\text{C}_6\text{H}_4\cdot\text{CHCl}\cdot[\text{CH}_2]_{n-3}}$, which is negligible when $n = 5$ and 6, is equivalent to 3·9 kcal. mole^{-1} when $n = 7$.

Dissociation of the cyanohydrins (33; R = Me, Et, Pr^i, Bu^t) of the ketones Ph·COR causes changes in intramolecular configuration resembling those in (31) and (32) and is therefore similarly affected by changes in intramolecular strain. The dissociation constants, 312, 148, 60, and 21 respectively, *decrease* with increase in the bulk of R.[71] The unusually high reactivity of the " secondary " hydrogen atoms of the α-methylene group of tetralin,[72] relatively to that of the " tertiary " hydrogen atom of cumene (34), towards peroxy-radicals is a further example of ready reaction caused by the reactant's having the conformation which is required for least potential energy in the transition state. Thus it is related to the ready ionisation of 1-chlorotetralin and the ready dissociation of the salts of 1 : 2 : 3 : 4-tetrahydroquinoline. The bond strength of a $C_{(1)}$–H bond of tetralin seems to be at least 2·2 kcal. mole^{-1} less than that of the tertiary C–H bond of cumene.[72] The relative rates of hydrogen abstraction from *cyclo*hexene, hept-3-ene,[73]

[70] G. Baddeley, J. Chadwick, and H. T. Taylor, *J.*, 1956, 448, 451.
[71] D. P. Evans and J. R. Young, *J.*, 1954, 1310.
[72] G. A. Russell, *J. Amer. Chem. Soc.*, 1955, **77**, 4583.
[73] E. C. Kooyman and A. Strang, *Rec. Trav. chim.*, 1953, **72**, 329.

and oct-1-ene,[74] and from 9 : 10-dihydroanthracene and diphenylmethane,[73] can be similarly explained.

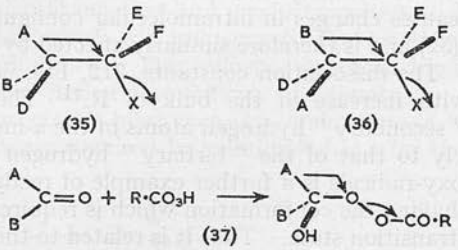


(33) (34)

The ultraviolet absorption spectra and rates of bromination of the methyl ethers of *o*-cresol and *m*-2-xylenol, the ethers *o*-C_6H_4·O·$[CH_2]_{n-3}$ and PhOR, where $n = 5$, 6, and 7, and R is Me, Et, Pr^i, and Bu^t, and the rates of solvolysis of their chloromethyl derivatives, show uniplanarity of the ether group and the benzene ring to be hindered in *m*-2-xylenol methyl ether, *tert.*-butoxybenzene, and homochroman (4 : 5 : 6 : 7-tetrahydrobenz[*b*]oxepin].[17] Care has to be exercised in comparing ultraviolet spectral relations with relations derived from rate and equilibrium data. In the last types, a re-orientation of atomic nuclei, both in the reacting molecules and in the surrounding solvent, takes place from ground state to transition state or product.

Course of reaction. Intramolecular steric strain can have an important influence on the rate and course of intramolecular rearrangements. Three aspects of its intervention are apparent in rearrangements of the type illustrated by the schemes (35) and (36).

(i) The approaches to the transition states for migration of A and B involve different increments of steric strain : B and E, and D and F, come into direct opposition in the migration of A; so do D and E, and A and F, in that of B. One reaction may therefore be more sterically hindered than the other. Recent examples of this type of discrimination include the nitrous acid deamination of β-amino-alcohols [75] and 20-amino-17-hydroxy-steroids.[76]



(35) (36) (37)

(ii) If the migrating group is aromatic, it must adopt a rotational conformation such that the π-electrons of the ring may effectively overlap the orbital which is made available by the departure of X. Steric interference

[74] L. Debiais, M. Niclause, and M. Letort, *Compt. rend.*, 1954, **239**, 539, 1040; L. Debiais, P. Horstman, M. Niclause, and M. Letort, *ibid.*, p. 587.

[75] D. Y. Curtin and M. C. Crew, *J. Amer. Chem. Soc.*, 1955, **77**, 354; D. Y. Curtin and S. Schumuckler, *ibid.*, p. 1105.

[76] F. Ramirez and S. Stafiej, *Chem. and Ind.*, 1955, 1180.

with this process will hinder the migration.[77] Thus, in the oxidation of *cyclo*hexyl phenyl ketone with per-acid, the *cyclo*hexyl group, which usually migrates comparatively seldom, migrates five times more readily than the phenyl group.[78]

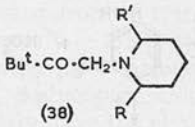
(iii) Steric hindrance by *ortho*-substituents is more apparent in pinacol rearrangements (35; X = D = OH) than in per-acid oxidation of *o*-substituted aromatic ketones (37); this suggests that steric interaction of the migrating group with the migration terminus may be involved.[79]

Steric acceleration. This consequence of steric interaction can arise in several ways. An obvious one is when steric strain is less in the transition state of reaction than in the reactant. Thus, it has been repeatedly argued,[80] is the comparatively high rate of solvolysis of highly branched tertiary alkyl halides to be explained. The failure of many of these halides to undergo measurable rearrangement during solvolysis indicates that " rearrangement forces " are negligible. The rates of solvolysis of the *p*-nitrobenzoates of highly branched *tert.*-alcohols in aqueous dioxan show no simple or regular variation with degree of branching, although the ester with the most crowded molecular structure is the most reactive.[81]

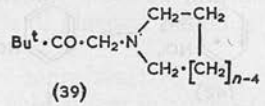
In alicyclic chemistry, the differences in the rates of solvolysis of cyclanyl toluene-*p*-sulphonates in acetic acid (*cyclo*hexyl 1, *cyclo*heptyl 31, *cyclo*decyl 539, *cyclo*undecyl 67, *cyclo*dodecyl 3·1, and *cyclo*eicosyl 1·9) are considered to be caused by changes in internal strain in passing to the transition state. Steric acceleration is apparent in the behaviour of the intermediate members.[82] The percentage of olefin which accompanies Kishner reduction of the cyclic α-amino-ketones:

$$\overbrace{\text{NMe}\cdot[\text{CH}_2]_{n-3}\cdot\text{CO}\cdot\text{CHMe}} \longrightarrow \overbrace{\text{NMe}\cdot[\text{CH}_2]_{n-3}\cdot\text{CH}_2\cdot\text{CHMe}} + \text{NHMe}\cdot[\text{CH}_2]_{n-3}\cdot\text{CH}{:}\text{CHMe}$$

increases in the order $n = 6 \ll 7 < 8$ and thus indicates that steric strain at the C_α–N bond increases in this order. Similar reduction [83] of the compounds (38) and (39) gives increasing amounts of *tert.*-butylethylene in the



(38)



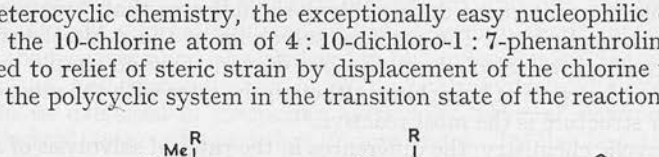
(39)

order (38), R = R' = H < R = Me, R' = H < R = R' = Me and (39), $n = 5 < 6 < 7$. In further illustration is the circumstance that the carbonyl groups of *cyclo*pentane- and *cyclo*hexane-1 : 2-dione are approximately parallel to one another and face in the same direction; it provides a degree of steric instability which is relieved by enolisation. This process is

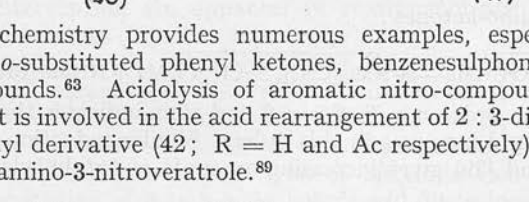
[77] C. K. Ingold, " Structure and Mechanism in Organic Chemistry," G. Bell and Sons Ltd., London, 1953, p. 478.
[78] S. L. Friess and N. Farnham, *J. Amer. Chem. Soc.*, 1950, **72**, 5518.
[79] W. H. Saunders, jun., *ibid.*, 1955, **77**, 4679.
[80] P. D. Bartlett and M. S. Swain, *ibid.*, p. 2801; H. C. Brown and M. Nakagawa, *ibid.*, p. 3610; H. C. Brown and Y. Okamoto, *ibid.*, p. 3619; H. C. Brown and I. Moritani, *ibid.*, p. 3623.
[81] P. D. Bartlett and M. Stiles, *ibid.*, p. 2806.
[82] R. Heck and V. Prelog, *Helv. Chim. Acta*, 1955, **38**, 1541.
[83] N. J. Leonard and S. Gelfand, *J. Amer. Chem. Soc.*, 1955, **77**, 3269.

aided also by hydrogen bonding of the adjacent oxygen atoms. These considerations do not apply to *cyclo*heptane-1 : 2-dione since its oxygen atoms are more widely separated; the difference is indicated by the ultraviolet absorption spectra and by the rates of halogenation of these diketones.[84] Again, the rearrangement of euphol (40) by acid is sterically accelerated. Rings B and C have the unfavourable conformation of two half-boats, which provides a conformational driving force for the acid-catalysed migration of methyl groups, to give a structure in which all three six-membered rings can adopt the chair conformation.[85] The alcoholysis of the toluene-*p*-sulphonate of *cis*-2-methyl*cyclo*pentanol is faster than that of the toluene-*p*-sulphonates of the *trans*-isomer and of *cyclo*pentanol; the difference is offered as evidence for steric acceleration. The same relation obtains between the corresponding *cyclo*hexane derivatives.[86]

In heterocyclic chemistry, the exceptionally easy nucleophilic displacement of the 10-chlorine atom of 4 : 10-dichloro-1 : 7-phenanthroline (41) is attributed to relief of steric strain by displacement of the chlorine from the plane of the polycyclic system in the transition state of the reaction.[87]



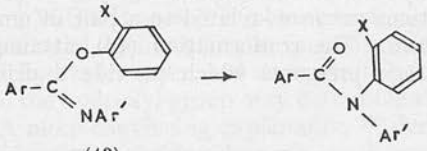
Aromatic chemistry provides numerous examples, especially in the acidolysis of *o*-substituted phenyl ketones, benzenesulphonic acids, and related compounds.[63] Acidolysis of aromatic nitro-compounds is not so well known; it is involved in the acid rearrangement of 2 : 3-dinitroaniline[88] and its *N*-acetyl derivative (42; R = H and Ac respectively), 2 : 3-dinitrophenol, and 4-amino-3-nitroveratrole.[89]



Another type of steric acceleration is illustrated by the influence of *o*-substituents on the Beckmann rearrangement of acetophenone oxime. They help to twist the α-hydroxyiminoethyl group out of the plane of the ring, so that the aryl group is correctly oriented for migration to the nitrogen

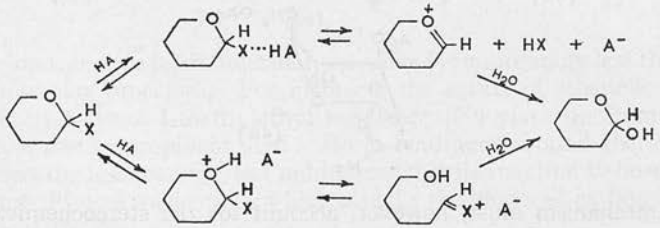
[84] G. Hesse and G. Krehbiel, *Chem. Ber.*, 1955, **593**, 35, 42.
[85] D. H. R. Barton, J. F. McGhie, M. K. Pradhan, and S. A. Knight, *J.*, 1955, 876.
[86] W. Hückel and H. D. Sauerland, *Annalen*, 1955, **592**, 190.
[87] R. A. Cutler and A. R. Surrey, *J. Amer. Chem. Soc.*, 1955, **77**, 2441.
[88] K. H. Pausacker and J. G. Scroggie, *J.*, 1955, 1897.
[89] K. C. Frisch, M. Silverman, and M. T. Bogert, *J. Amer. Chem. Soc.*, 1943, **65**, 2432.

atom.[90] The accelerating influence of *o*-substituents on the rate of thermal intramolecular rearrangement of substituted phenyl *N*-phenylbenzimidoates (43) is largely an entropy effect; seemingly, an *o*-substituent hinders rotation of the phenyl group and thereby lessens the entropy decrease in going from the reactant to a transition state which requires restriction of this mode of rotation.[91]



(43)

Reaction at Position 1 of Glycosides.—During 1955 there have been several attempts to provide a consistent interpretation of the relative rates of reaction at position 1 of glycosides; all are based on steric consideration. Substitution is of the type S_N1 and subject to electrophilic catalysis; thus the logarithm of the first-order rate constant for the hydrolysis of these compounds with acid varies linearly with the acidity function H_0; the reaction involves fission of the hexose–oxygen bond.[92] There are two possible formulations of the mechanism (as annexed). However, this mechan-



istic ambiguity does not obtain in all instances: *e.g.*, while the acid-base-catalysed mutarotation of tetra-*O*-methylglucose in nitromethane[93] takes the lower course (X = OH), solvolysis of glucosyl halide is more likely to occur by the other (X = halogen).

In general, β-glycopyranosides are more reactive than the α-anomers. The latter usually have the glycosidic substituent in an axial position[94] and it has been suggested that the axial α-glycosidic substituent is *shielded* from the electrophilic catalyst by substituents in the pyran ring. The concept of shielding has also been employed[95] to account for the greater stability of normal than of 2-deoxypyranosides. More convincing explanations have been based on the well-known principles of conformational analysis:

[90] D. E. Pearson and E. D. Watts, *J. Org. Chem.*, 1955, **58**, 494; D. E. Pearson and W. E. Cole, *ibid.*, 1955, **58**, 488; K. von Auwers, M. Lechner, and H. Bundersmann, *Ber.*, 1925, **58**, 36.

[91] K. B. Wiberg and B. I. Rowland, *J. Amer. Chem. Soc.*, 1955, **77**, 2205.

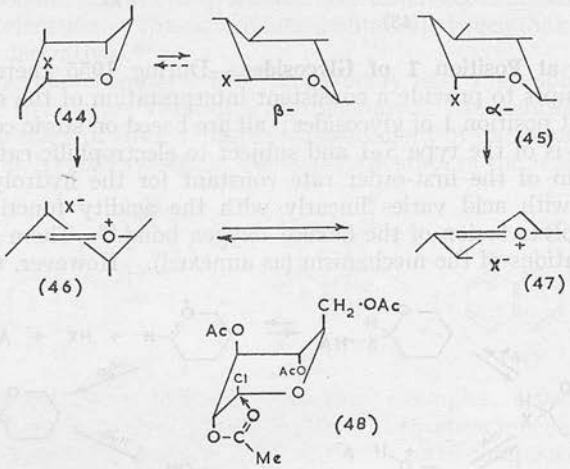
[92] C. A. Bunton, T. A. Lewis, D. R. Llewellyn, H. Tristram, and C. A. Vernon, *Nature*, 1954, **174**, 560.

[93] A. M. Eastham, E. L. Blackall, and G. A. Latremouille, *J. Amer. Chem. Soc.*, 1955, **77**, 2182; E. L. Blackall and A. M. Eastham, *ibid.*, p. 2184.

[94] For examples see S. A. Barker, E. J. Bourne, and M. Stacey, *J.*, 1954, 171; S. A. Barker, E. J. Bourne, K. Stephens, and D. H. Whiffen, *J.*, 1954, 3468.

[95] G. N. Richards, *Chem. and Ind.*, 1955, 228.

dissociation of the $C_{(1)}$–X bond, the rate-determining step, will be accompanied by a change of the pyranoside ring from a chair to a half-chair form, and its rate will therefore be influenced by the extent to which the substituents at positions 2—5 help or hinder this change of conformation.[96] It is reasonable to assume that the β-anomer in the conformation (44) dissociates to the ion (46) whereas the α-anomer (45) gives (47); for in (44) and (45) the chlorine atoms are *trans*-related to a pair of unshared electrons of the ring-oxygen atom. The conformation (44), attained through kinetic disturbance, has steric pressures which provide a driving force for the dissociation.[97]



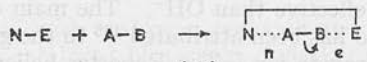
The mechanism must, however, account for the stereochemical consequences as well as for the relative rates of reaction. Reaction of the ions or ion-pairs with a solvent molecule or other reagent may be influenced by shielding by the anion and by the substituents in the pyran ring, and by change of intramolecular strain as the half-chair changes into a chair or a boat conformation. When 3 : 4 : 6-tri-*O*-acetyl-β-D-glucosyl chloride is dissolved in acetic acid the chlorine atom is replaced by acetate with a strong tendency for inversion. The corresponding α-D-glucosyl chloride is a hundred times less reactive, and solvolysis still proceeds with extensive inversion.[97] The greater difference in reactivity between the anomeric D-glycopyranose penta-acetates [98] and tetra-*O*-acetylglycosyl chlorides (it can be as great as 450) is shown to be caused mainly by anchimeric assistance to dissociation at $C_{(1)}$ by the 2-acetoxy-group in the case of the 1 : 2-*trans*-β-anomer [99] (see 48).

Oxidation of saccharides with bromine is believed [100] to be initiated by

[96] J. T. Edward, *Chem. and Ind.*, 1955, 1102; G. Huber, *Helv. Chim. Acta*, 1955, **38**, 1224.
[97] R. U. Lemieux and G. Huber, *Canad. J. Chem.*, 1955, **33**, 128.
[98] R. U. Lemieux and C. Brice, *ibid.*, p. 109.
[99] R. U. Lemieux, C. Brice, and G. Huber, *ibid.*, p. 134; R. U. Lemieux, W. P. Shyluk, and G. Huber, *ibid.*, p. 148.
[100] R. Bentley, *Nature*, 1955, **176**, 870.

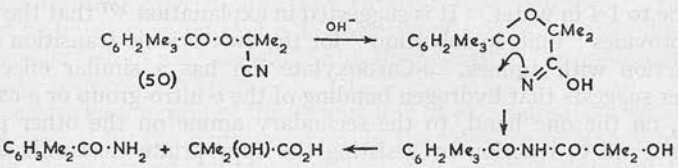
attack by the oxidant at the 1-hydroxyl group. Readier access to an equatorial than to an axial hydroxyl group provides a plausible basis for an explanation of the readier oxidation of the α-anomers. However, the rate of oxidation of [1-^{3}H]ethanol with bromine is 0·57 that of ethanol, and this ratio together with the reaction kinetics is offered [101] as evidence for a mechanism involving the transfer of hydride ion from methylene-carbon to bromine. Oxidation, >CH·OH ⟶ >C=O by chromic acid is usually faster with axial than equatorial hydroxyl; the rate-determining step involves removal of the C–H hydrogen atom, and access to this atom (equatorial > axial) rather than to the hydroxyl group may determine the relative rates of these oxidations. A more convincing explanation [102] derives from recognition that the rate-determining step involves change of tetrahedral to trigonal carbon and may be sterically accelerated by a release of strain which is greater when the hydroxyl group of the initial alcohol is axial than when it is equatorial.

Association of Reactants.—Application of the concept of association of reactants to the synchronisation of electrophilic (e) and nucleophilic (n) attack (49) was reported last year; its importance cannot be over-emphasised and is further illustrated in this Report.



(49)

Steric hindrance to intramolecular processes is considerably less than that to intermolecular processes. For instance, the action of ethanolic sodium hydroxide on 1-cyano-1-methylethyl mesitoate (50) gives mesitamide and α-hydroxy-α-methylpropionic acid: steric hindrance around the carbonyl group allows the less reactive, but unhindered, nitrile function to be attacked and converted into a nucleophile which attacks the hindered carbonyl group intramolecularly.[103]

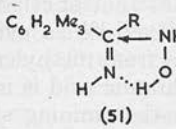


(50)

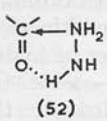
Mesityl methyl and mesityl phenyl ketimine react with hydroxylamine to give ketoximes whereas the corresponding ketones do not.[104] The Reporter suggests that the ketimines, because of their greater basic strength, are the better able to associate with the reagent and thus to effect intramolecular rearrangement (51). Similarly, the efficacy of anhydrous conditions in the reduction of hindered ketones with hydrazine is related to the association of the reactants (52): a component with an acid strength greater than that of hydrazine, *e.g.*, water, would displace the hydrazine molecule from the carbonyl-oxygen atom. Again, the specific accelerating

[101] L. Kaplan, *J. Amer. Chem. Soc.*, 1954, **76**, 4645.
[102] J. Schreiber and A. Eschenmoser, *Helv. Chim. Acta*, 1955, **38**, 1529.
[103] J. P. Freeman and G. B. Lucas, *J. Amer. Chem. Soc.*, 1955, **77**, 2334.
[104] C. R. Hauser and D. S. Hoffenberg, *ibid.*, p. 4885.

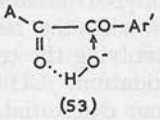
influence of hydroxide ion (cf. MeO^- and PhO^-) on the rate of benzilic rearrangements [105] can be attributed to ready nucleophilic attack (the rate-determining process) provided by hydrogen bonding (53).



(51)



(52)



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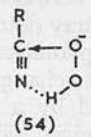
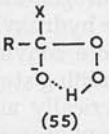


(54)



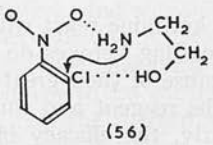
(55)

The view that hydrogen bonding aids the formation of five-membered chelate rings finds further application in the interpretation of the accelerating influence of hydrogen peroxide on the rate of alkaline hydrolysis of nitriles:

$$R{\cdot}CN + HO_2^- \longrightarrow R{\cdot}C(O{\cdot}OH){:}N^- \xrightarrow{H_2O_2} O_2 + OH^- + R{\cdot}CO{\cdot}NH_2$$

HO_2^- is 10^4 times more effective than OH^-. The main difference is in the entropy of activation and has been attributed [106] to the greater nucleophilic strength of the hydroperoxide ion. The Reporter believes that, as in the previous instances, the difference finds its interpretation in the contribution of hydrogen bonding (54); this helps to bring together the reacting groups. Hydroperoxide ion reacts with acid halides and phenyl esters but not with alkyl esters; dissociation of the C–X bond in (55) seems to require the basic strength of X^- to be less than that of $HO{\cdot}O^-$.

Whereas *p*-chloronitrobenzene is usually the more reactive towards anionic reagents (*e.g.*, MeO^- and PhS^-), the *o*-isomer is the more reactive towards amines; when the amine is piperidine, the *o*/*p* ratio changes from 50 in xylene to 1·4 in water. It is suggested in explanation [107] that the *o*-nitro-group provides " built-in solvation " for the zwitterionic transition state of the reaction with amines. *o*-Carboxylate ion has a similar effect. The Reporter suggests that hydrogen bonding of the *o*-nitro-group or *o*-carboxylate ion, on the one hand, to the secondary amine on the other provides additional aid to reaction by assisting the appropriate electronic displace-



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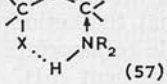


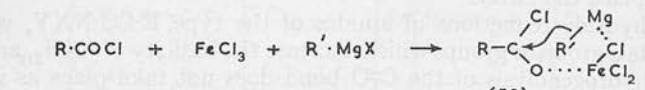
(57)

ments and by helping to bring together the reacting groups. Ethanolamine is extraordinarily reactive towards *o*-chloronitrobenzene; [108] the explanation is apparent in the formulation (56). The reaction of a tertiary amine with

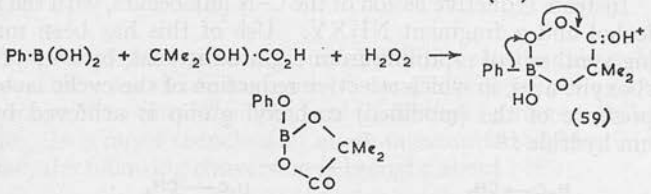
[105] M. T. Clark, E. C. Hendley, and O. K. Neville, *J. Amer. Chem. Soc.*, 1955, **77**, 3280.
[106] K. B. Wiberg, *ibid.*, p. 2519.
[107] J. F. Bunnett and R. J. Morath, *ibid.*, p. 5051; J. F. Bunnett, R. J. Morath, and T. Okamoto, *ibid.*, p. 5055; J. F. Bunnett and R. F. Snipes, *ibid.*, p. 5422.
[108] P. L. Gordon, T. A. Alfrey, jun., and E. I. Becker, *J. Phys. Chem.*, 1955, **59**, 583.

1-methylallyl chloride proceeds simultaneously through the normal (S_N2) and the abnormal (S_N2') displacement; therefore the latter process does not require hydrogen-bonding of the entering and leaving groups. It seems likely, however, that such bonding (57) promotes S_N2' displacements and thus makes them more important when the amine is secondary.[109]

Association of reactants may involve a third component. Thus $FeCl_3$, $MgBr_2$, BF_3, $MnCl_2$, etc., catalyse the interaction (58) of acid chloride and Grignard reagent.[110] An interesting characteristic of the reactions of



(58)



(59)

benzeneboronic acid with hydrogen peroxide and halogen severally is the fact that they are catalysed by certain chelating agents; *e.g.*, α-hydroxy-α-methylpropionic acid catalyses the reaction with hydrogen peroxide.[111] The Reporter suggests that a cyclic intermediate (59) is formed.

G. B.

3. GENERAL METHODS.

Reduction.—*Catalytic hydrogenation.* The predominant catalytic hydrogenation of Δ^4- and Δ^5-steroids to give A/B-*cis*- and A/B-*trans*-products, respectively, has been explained by conformational analysis of the possible complexes formed between substrate, catalyst, and hydrogen.[1] However, it should be borne in mind that a 3α-substituent exerts a profound effect on the hydrogenation of a Δ^5-steroid;[2] and the acidity of the medium has also to be taken into consideration.

The selective reduction of the olefinic link of an αβ-unsaturated aldehyde can be achieved by the hydrogenation, in the presence of Raney nickel, of the Schiff's base formed (without isolation) with 2-aminobutan-1-ol, followed by hydrolysis.[3] It appears likely that other amines could be used.

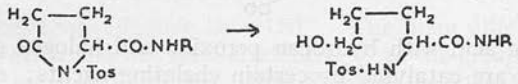
For the hydrogenation of carboxylic acids to primary alcohols[4] and of aliphatic amides to primary amines,[5] the use of ruthenium or of copper–chromium oxide catalysts is recommended. A novel method for the conversion of a nitrile (such as benzyl cyanide) into the corresponding aldehyde

[109] W. G. Young, R. A. Clement, and C.-H. Shih, *J. Amer. Chem. Soc.*, 1955, **77**, 3061.
[110] V. Franzen and H. Krauch, *Chem. Ztg.*, 1955, **79**, 137.
[111] H. G. Kuivila and R. A. Wiles, *J. Amer. Chem. Soc.*, 1955, **77**, 4830.
[1] H. I. Hadler, *Experientia*, 1955, **11**, 175.
[2] J. R. Lewis and C. W. Shoppee, *J.*, 1955, 1365; *Ann. Reports*, 1953, **50**, 167.
[3] E. P. Goldberg and H. R. Nace, *J. Amer. Chem. Soc.*, 1955, **77**, 359.
[4] J. E. Carnahan, T. A. Ford, W. F. Gresham, W. E. Grigsby, and G. F. Hager, *J. Amer. Chem. Soc.*, 1955, **77**, 3766; A. Guyer, A. Bieler, and M. Sommaruga, *Helv. Chim. Acta*, 1955, **38**, 976.
[5] A. Guyer, A. Bieler, and G. Gerliczy, *Helv. Chim. Acta*, 1955, **38**, 1649.

consists of hydrogenation (Raney nickel) in the presence of semicarbazide. The aldehyde is thus trapped as the semicarbazone, which is relatively stable to further reduction and can be decomposed with formaldehyde.[6]

Lithium aluminium hydride. New uses for this reagent continue to appear, although interest is spreading to many other hydrides, particularly those whose action is more selective and those which are cheaper to prepare. Thus, the reactions of sodium aluminium hydride appear to differ little from those of the lithium compound, and it seems that the former reagent might replace the latter.[7]

The hydride reductions of amides of the type R·CO·NXY, where XY represents a group or groups which decrease the basicity of $-NH_2$, are unusual in that hydrogenolysis of the C=O bond does not take place as in normal amides. Instead, reductive fission of the C–N link occurs, with the formation of an alcohol and a fragment NHXY. Use of this has been made in an interesting synthesis of L-proline from L-glutamic acid, by way of L-pyrrolidonecarboxylic acid, in which selective reduction of the cyclic lactone group in the presence of the (modified) carboxyl group is achieved by lithium aluminium hydride : [8]



(R = H or Ph; Tos = *p*-$C_6H_4Me{\cdot}SO_2$)

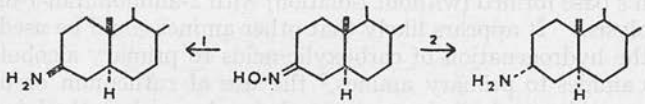
It is possible, in a smooth reaction, to reduce *N*-alkyl oximes [9] to *NN*-dialkylhydroxylamines (compounds which have also been obtained by the pyrolysis of *tert.*-amine oxides) : [10]

$$RR'{\cdot}N(\rightarrow O){\cdot}R'' \longrightarrow RR'CH{\cdot}N(OH){\cdot}R''$$

The reduction of *O*-alkyl oximes leads to fission of the molecule : [9]

$$RR'C{:}N{\cdot}OR'' \longrightarrow RR'CH{\cdot}NH_2 + R''OH$$

In contrast to reduction with sodium and alcohol (which gives an equatorial amine), the action of lithium aluminium hydride on cholestan-3-one oxime yields predominantly the axial amine, thus resembling catalytic hydrogenation in acidic media:

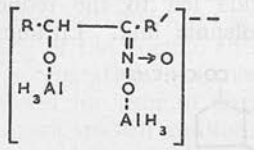
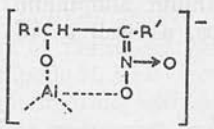


Reagents : 1, Na–EtOH. 2, $LiAlH_4$.

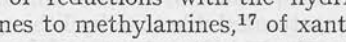
Similar results are obtained with coprastan-3-one oxime.[11]

[6] H. Plieninger and G. Werst, *Angew. Chem.*, 1955, **67**, 156.
[7] A. E. Finholt, E. C. Jacobson, A. E. Ogard, and R. Thompson, *J. Amer. Chem. Soc.*, 1955, **77**, 4163.
[8] Z. Pravda and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1955, **20**, 1.
[9] O. Exner, *ibid.*, p. 202.
[10] M. A. T. Rogers, *J.*, 1955, 769.
[11] D. E. Evans, C. W. Shoppee, and G. H. R. Summers, *Chem. and Ind.*, 1954, 1536; L. Lábler, V. Černý, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1954, **19**, 1249.

Fission of a C–C bond, which often occurs in the reduction of β-nitro-alcohols, can be largely avoided by (*A*) adding the hydride to the nitro-compound, rather than *vice versa* (*B*). This is explained by the instability of the doubly charged ion (1), compared with the singly charged ion (2) which would be formed in *B* and *A*, respectively. The low ether-solubility of the complex appears to be not unfavourable to reduction (in competition with fission), for the use as solvent of tetrahydrofuran, in which the complex is soluble, does not increase the yield.[12]

(1)   (2)

Examples of selective reductions with lithium aluminium hydride at low temperatures are of aliphatic nitro-esters, containing *sec.*-, *tert.*-, or *gem*-dinitro-groups,[13] to nitro-alcohols, and of methyl hydrogen phthalate [14] to phthalide. In a novel reduction of an αβ-unsaturated carboxylic ester, *apo*-yohimbine, the following conversion is brought about : [15]



Other examples of reductions with the hydride are of ozonides to alcohols,[16] of urethanes to methylamines,[17] of xanthates to thiols,[18] and of butenolides.[19]

As has been already noted,[20] the reducing properties of lithium aluminium hydride are modified by the addition of aluminium chloride, which presumably results in the formation of AlH_3, AlH_2Cl, and $AlHCl_2$. In contrast to earlier findings,[21] such a solution does not reduce aromatic nitro-compounds to amines.[22] Nitriles, on the other hand, give good yields of amines, and certain aromatic nitro-aldehydes are reduced to nitro-alcohols, while bromo-acid chlorides and bromo-ketones yield bromohydrins.

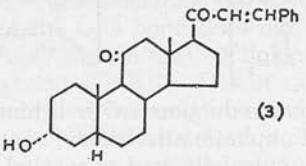
Borohydrides of the alkali metals. The study of the selective reduction of steroidal ketones with these reagents continues to give profitable results. It can be briefly stated that the reactivities of such compounds towards sodium borohydride decrease in the order : 3-one > 17- and 20-one > 4-en-3-one > 11-one. With the exception of the last (in the case of which steric effects probably predominate), the above sequence is paralleled by the order of decreasing oxidation potentials of simple analogues.[23]

[12] A. Dornow and M. Gellrich, *Annalen*, 1955, **594**, 177.
[13] H. Feuer and T. J. Kucera, *J. Amer. Chem. Soc.*, 1955, **77**, 5740.
[14] E. L. Eliel, A. W. Burgstahler, D. E. Rivard, and L. Haefele, *ibid.*, p. 5092.
[15] J. Brüesch and P. Karrer, *Helv. Chim. Acta*, 1955, **38**, 905.
[16] F. L. Greenwood, *J. Org. Chem.*, 1955, **20**, 803.
[17] R. L. Dannley, M. Lukin, and J. Shapiro, *ibid.*, p. 92; J. Knabe, *Arch. Pharm.*, 1955, **288**, 469.
[18] C. Djerassi, M. Gorman, F. X. Markley, and E. B. Oldenburg, *J. Amer. Chem. Soc.*, 1955, **77**, 568.
[19] F. Ramierez and M. B. Rubin, *ibid.*, pp. 2905, 3768.
[20] B. R. Brown, *J.*, 1952, 2756.
[21] F. Wiberg and A. Jahn, *Z. Naturforsch.*, 1952, **7b**, 580.
[22] R. F. Nystrom, *J. Amer. Chem. Soc.*, 1955, **77**, 2544.
[23] J. K. Norymberski and G. F. Woods, *J.*, 1955, 3426; *Ann. Reports*, 1952, **49**, 142; 1954, **51**, 227.

Contrary to an earlier report,[24] lactose and several other sugars can be reduced almost quantitatively to the corresponding glycitols by sodium borohydride.[25]

The reagent brings about the replacement of bromine by hydrogen in aliphatic perbromonitro-compounds,[26] and the reduction [27] of carbinolamine bases, $>N{\cdot}CH(OH)- \longrightarrow >N{\cdot}CH_2-$.

A comparative study of the reduction of an unsaturated diketone (3) with three different hydrides illustrates the different reducing power of these reagents.[28] Lithium aluminium hydride led to the reduction of both carbonyl groups, as well as of the olefinic link. Lithium borohydride



(3)

reduced the two carbonyl groups, without affecting the olefinic link (the yield was low), while potassium borohydride reduced only the 20-carbonyl group. An interesting point is that further reduction of the last product by lithium aluminium hydride led to the reduction of the other carbonyl group and the olefinic link, but the product was the 20-epimer of that obtained by direct reduction of the unsaturated diketone by lithium aluminium hydride.

Sodium trimethoxyborohydride [29] and lithium borohydride [30] have both been used to reduce carboxylic chlorides to primary alcohols, without affecting ester groups present in the same molecule.[31]

Aluminium and calcium borohydrides. Calcium borohydride has been used for the reduction of a considerable number of esters to primary alcohols in high yield.[32] It is selective in its action so that, for example, ethyl *p*-nitrobenzoate can be reduced to *p*-nitrobenzyl alcohol in 96% yield. Azlactones are also reduced. A solution of the reagent in tetrahydrofuran can be prepared by treatment of one of calcium iodide with sodium borohydride. An alcoholic solution can also be used for reductions, provided the temperature is kept below −10° to avoid alcoholysis of the hydride.

The addition of aluminium chloride to a solution of sodium borohydride in diethylene glycol dimethyl ether gives a clear solution, without the precipitation of sodium chloride, so it was thought unlikely that aluminium borohydride would be present.[33] This solution rapidly reduces carboxylic esters and nitriles; but nitro- and amido-groups are not affected. It is interesting that alk-1-enes are reduced at 75°, although unconjugated alk-2-enes are unchanged under these conditions. Other workers [34] also found

[24] L. Hough, J. K. N. Jones, and E. L. Richards, *Chem. and Ind.*, 1953, 1064.
[25] W. J. Whelan and K. Morgan, *ibid.*, 1955, 1449.
[26] K. Klager, *J. Org. Chem.*, 1955, **20**, 647.
[27] S. Bose, *J. Indian Chem. Soc.*, 1955, **32**, 450.
[28] E. P. Oliveto, C. Gerold, and E. B. Hershberg, *J. Amer. Chem. Soc.*, 1954, **76**, 6111.
[29] H. C. Brown and E. J. Mead, *ibid.*, 1953, **75**, 6263.
[30] J. Kollonitsch, O. Fuchs, and V. Gábor, *Nature*, 1954, **173**, 125.
[31] W. Fuchs, *Chem. Ber.*, 1955, **88**, 1825.
[32] J. Kollonitsch, O. Fuchs, and V. Gábor, *Nature*, 1955, **174**, 346.
[33] H. C. Brown and B. C. S. Rao, *J. Amer. Chem. Soc.*, 1955, **77**, 3164.
[34] J. Kollonitsch and O. Fuchs, *Nature*, 1955, **176**, 1081.

that sodium borohydride does not react with aluminium chloride in tetrahydrofuran solution, although calcium borohydride gives a good yield of aluminium borohydride :

$$2AlCl_3 + 3Ca(BH_4)_2 \longrightarrow 2Al(BH_4)_3 + 3CaCl_2$$

In this case, calcium chloride is precipitated and the hydride left in solution. Such solutions, and also those prepared by dissolving distilled aluminium borohydride [35] in tetrahydrofuran, showed properties very similar to those mentioned above [33] for the solution prepared from sodium borohydride.

Dialkylaluminium hydrides and aluminium alkyls. As part of his extensive study of the organic chemistry of aluminium may be mentioned here Ziegler's preparation [36] of these compounds, which promise to become cheap substitutes for lithium aluminium hydride, although experimental details of the work are still awaited. These compounds are prepared by the interaction of aluminium, hydrogen, and alkenes, under certain conditions :

$$2Al + 6C_2H_4 + 3H_2 \longrightarrow 2Al(C_2H_5)_3$$
$$4Al(C_2H_5)_3 + 2Al + 3H_2 \longrightarrow 6AlH(C_2H_5)_2$$

The dialkylaluminium hydrides lead to the same products of reduction of organic compounds as lithium aluminium hydride; but normally only the Al–H bond reacts. Thus, to reduce esters to alcohols, two molecules of reagent are required for each ester molecule. On the other hand, the *iso*butyl compounds Bu^i_2AlH and Bu^i_3Al react towards certain aldehydes simply as a mixture of *iso*butene and aluminium hydride, *i.e.*, one molecule of reagent reduces three molecules of aldehyde. The latter reagents react smoothly with chloral and with aromatic and unsaturated aldehydes; in contrast, triethylaluminium, which had been prepared much earlier (by the interaction of a Grignard reagent with aluminium chloride),[37] is not generally a good reducing agent for aldehydes, although it does reduce chloral in good yield to the corresponding alcohol.[38]

Alkali metals. The discovery of a mild method for the reduction of phenols to aromatic hydrocarbons fills a gap in reduction methods, even though it is not very promising for dihydric phenols.[39] This method, which gives yields around 75% in the benzene and naphthalene series, consists in the reduction by lithium or sodium in liquid ammonia of an aryl diethyl phosphate. The latter may be made by the addition of triethylamine to a carbon tetrachloride solution of the phenol and diethyl phosphite (prepared directly from ethanol and phosphorus trichloride) or by treating an aqueous solution of the sodium phenoxide with tetraethyl pyrophosphate :

$$ArOH + HO{\cdot}P(OEt)_2 + CCl_4 + NEt_3 \longrightarrow ArO{\cdot}PO(OEt)_2 + CHCl_3 + NHEt_3\}Cl$$
$$ArO{\cdot}PO(OEt)_2 + 2M + NH_3 \longrightarrow ArH + M{\cdot}NH_2 + MO{\cdot}PO(OEt)_2$$

Aromatic hydrocarbons undergo selective reduction to mono-olefins by lithium in aliphatic amines of low molecular weight.[40] Thus naphthalene

[35] H. I. Schlesinger, H. C. Brown, and E. K. Hyde, *J. Amer. Chem. Soc.*, 1953, **75**, 209.
[36] K. Ziegler, H. G. Gellert, K. Zosel, W. Lehmkuhl, and W. Pfohl, *Angew. Chem.*, 1955, **67**, 424; K. Ziegler, K. Schneider, and J. Schneider, *ibid.*, p. 425.
[37] E. Krause and B. Wendt, *Ber.*, 1923, **56**, 466.
[38] H. Meerwein, G. Hinz, H. Majert, and H. Sönke, *J. prakt. Chem.*, 1937, **147**, 226.
[39] G. W. Kenner and N. R. Williams, *J.*, 1955, 522.
[40] R. A. Benkeser, R. E. Robinson, D. M. Sauve, and O. H. Thomas, *J. Amer. Chem. Soc.*, 1955, **77**, 3230; R. A. Benkeser, C. Arnold, R. F. Lambert, and O. H. Thomas, *ibid.*, p. 6042.

and tetralin are reduced to Δ^9-octalin, accompanied by only a trace of the $\Delta^{1:9}$-isomer. It is suggested that the process involves a rapid 1 : 4-addition of lithium, reaction of the product with the solvent, isomerisation of the resulting 1 : 4-dihydro-compound to give a conjugated system, and further 1 : 4-reduction. The final 1 : 2-reduction is slow, thus permitting good yields of mono-olefins to be obtained. By this method of reduction, acetylenes give *trans*-olefins.[41] Further work on the Birch reduction of aromatic hydrocarbons has been reported.[42]

The direct, stereospecific conversion of undeca-1 : 7-diyne into *trans*-undeca-7-en-1-yne in 75% yield has been attained by protection of the 1-ethynyl group as the sodium salt by reaction with sodamide in liquid ammonia, before reduction with sodium in the same medium.[43]

The difficulty of releasing the sugar for identification from pyrimidine nucleosides has been circumvented by Birch reduction of the heterocyclic ring, after which hydrolysis can be readily effected.[44]

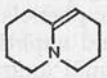
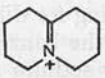
Other methods. The reducing power of the system used in the Huang-Minlon modification of the Wolff–Kishner reduction procedure is increased if water is rigorously excluded, thus permitting the reduction of sterically hindered ketones.[45]

Cumulenes, including (4), have been obtained in high yield by the action of stannous chloride on acetylene-, diacetylene-, and triacetylene-diol.[46]

(4) PhHC:C:C:C:C:CHPh

Treatment of an acyloin with propane-1 : 3-dithiol in the presence of zinc chloride and hydrogen chloride in benzene yields a mercaptal in which the hydroxyl group has been replaced by hydrogen.[47]

Dehydrogenation.—The dehydrogenation[48] of octahydropyridocoline with mercuric acetate in dilute acetic acid gives the hexahydro-derivative (5), in which the position of the double bond was shown spectroscopically.[49] In the salts of this product, the double-bond moves to give the 5 : 10-unsaturated compound (6).

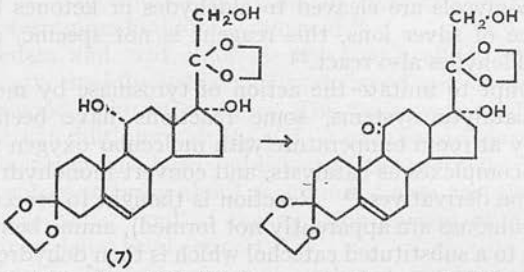
(5)   (6)

Polycyclic, aromatic hydrocarbons have been obtained[50] by vapour-phase dehydrogenation of *cyclo*alkanes with 9 or 12—18 ring members, in presence of palladium–charcoal at 400°.

The conversion of benzyl alcohols into benzaldehydes by various reagents has been described. The use of dinitrogen tetroxide in chloroform or carbon tetrachloride at ordinary temperatures gives excellent yields even when

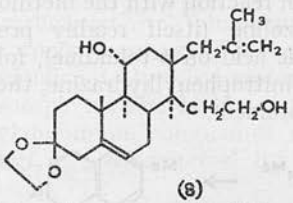
[41] R. A. Benkeser, G. Schroll, and D. M. Sauve, *J. Amer. Chem. Soc.*, **77**, 1955, 3378.
[42] W. Hückel and U. Wörffel, *Chem. Ber.*, 1955, **88**, 338.
[43] N. A. Dobson and R. A. Raphael, *J.*, 1955, 3558.
[44] D. C. Burke, *J. Org. Chem.*, 1955, **20**, 643.
[45] D. H. R. Barton, D. A. J. Ives, and B. R. Thomas, *J.*, 1955, 2056.
[46] R. Kuhn and H. Krauch, *Chem. Ber.*, 1955, **88**, 309.
[47] D. J. Cram and M. Cordon, *J. Amer. Chem. Soc.*, 1955, **77**, 1810.
[48] N. J. Leonard, A. S. Hay, R. W. Fulmer, and V. W. Gash, *ibid.*, p. 439; N. J. Leonard, P. D. Thomas, and V. W. Gash, *ibid.*, p. 1552.
[49] N. J. Leonard and D. M. Locke, *ibid.*, p. 437.
[50] V. Prelog, V. Boarland, and S. Polyák, *Helv. Chim. Acta*, 1955, **38**, 434.

ortho-substituents are present.[51] Manganese dioxide [52] in chloroform, ether or hexane yields up to 89%, and oxygen with platinum in ethyl acetate, water, or dilute acetone, up to 72% of the desired product.[53] The last reagent is also effective for the dehydrogenation of other primary alcohols and of secondary alcohols. In the steroid series, like *tert.*-butyl hypochlorite,[54] it appears to convert a 3-hydroxyl group selectively into a keto-group. The yields are claimed to be superior to those obtained by the Oppenauer method; and the specificity of attack at the 3-position contrasts with the preferential attack at other positions with chromic acid or *N*-bromosuccinimide. The reaction, however, failed with cholesterol. Chromium trioxide in pyridine has, in the case of 11-*epi*hydrocortisone 3 : 20-bisethylene ketal (7), brought about selective oxidation of the hydroxyl group at position 11 to a keto-group, thus leading to a convenient synthesis of hydrocortisone.[55]



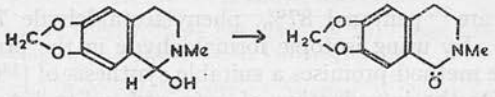
(7)

In the case of a tricyclic diol (8), however, it caused dehydrogenation of both the primary and the secondary alcoholic group.[56]

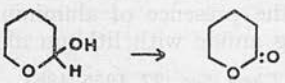


(8)

Manganese dioxide [52] has been used to bring about the conversions (9) ⟶ (10) and (11) ⟶ (12).



(9) (10)



(11) (12)

[51] B. O. Field and J. Grundy, *J.*, 1955, 1110.
[52] R. J. Highet and W. C. Wildman, *J. Amer. Chem. Soc.*, 1955, **77**, 4399.
[53] R. P. A. Sneeden and R. B. Turner, *ibid.*, pp. 130, 190.
[54] G. S. Fonken, J. L. Thompson, and R. H. Levin, *ibid.*, p. 172.
[55] W. S. Allen, S. Bernstein, and R. Littell, *ibid.*, 1954, **76**, 6116.
[56] G. I. Poos, W. F. Johns, and L. H. Sarett, *ibid.*, 1955, **77**, 1026.

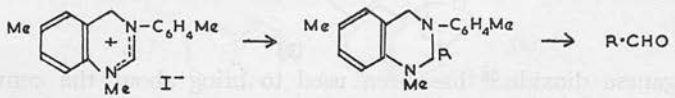
N-Bromosuccinimide converts the acetals of α-keto-aldehydes into α-keto-esters.[57]

Oxidation.—Peroxytrifluoroacetic acid [58] appears to be a promising reagent, not only for the preparation of α-glycols from alkenes,[59] but also for the epoxidation of alkenes.[58] Reaction is carried out in methylene dichloride solution, in the presence of sodium carbonate or disodium hydrogen phosphate, which removes the (much stronger) trifluoroacetic acid produced. High yields of epoxides remain in the solution. Terminal alkenes, such as oct-1-ene, which give low yields with peracetic acid, here give excellent yields. In contrast to the action of other peracids on olefins carrying negative substituents, the reaction goes well with methyl methacrylate. The reagent is also suitable for the Baeyer–Villiger oxidation of cyclic ketones [60] and similar reactions.[61] Oximes are oxidised to nitro-alkanes.[62]

Although α-glycols are cleaved to aldehydes or ketones by persulphate in the presence of silver ions, this reagent is not specific, as monohydric alcohols and aldehydes also react.[63]

In an attempt to imitate the action of tyrosinase by means of simple, homogenous catalytic systems, some reactions have been found which proceed rapidly at room temperature with molecular oxygen as oxidant and copper–amine complexes as catalysts, and convert monohydric phenols into amino-*o*-quinone derivatives.[64] Reaction is thought to proceed through the *o*-quinone (*p*-quinones are apparently not formed), amine being then rapidly added, leading to a substituted catechol which is then dehydrogenated to the corresponding *o*-quinone.

Carbonyl Compounds.—A new method for converting Grignard reagents into aldehydes consists in reaction with the methiodide (13) of 3 : 4-dihydro-6-methyl-3-*p*-tolylquinazoline (itself readily prepared by the action of formaldehyde and formic acid on *p*-toluidine), followed by acid hydrolysis in the presence of 2 : 4-dinitrophenylhydrazine, the corresponding derivative of the aldehyde being obtained : [65]



(13)

Typical yields are : pentanal 87%, phenylacetaldehyde 74%, and benzaldehyde 95%. By using isotopic formaldehyde in the preparation of the quinazoline, the method promises a suitable synthesis of [^{14}C]aldehydes.

A method for the introduction of a formyl group into the nucleus of aromatic hydrocarbons and aryl ethers consists in reaction with *N*-methylcarbaniloyl chloride in the presence of aluminium chloride, followed by reduction of the resulting amide with lithium aluminium hydride, in the

[57] J. B. Wright, *J. Amer. Chem. Soc.*, **77**, 1955, 4883.
[58] W. D. Emmons and A. S. Pagano, *ibid.*, p. 89.
[59] W. D. Emmons, A. S. Pagano, and J. P. Freeman, *ibid.*, 1954, **76**, 3472.
[60] W. F. Sager and A. Duckworth, *ibid.*, 1955, **77**, 188.
[61] W. D. Emmons and G. B. Lucas, *ibid.*, p. 2287.
[62] W. D. Emmons and A. S. Pagano, *ibid.*, p. 4557.
[63] F. P. Greenspan and H. W. Woodburn, *ibid.*, 1954, **76**, 6345.
[64] W. Brackman and E. Havinga, *Rec. Trav. chim.*, 1955, **74**, 937, 1021, 1070.
[65] H. M. Fales, *J. Amer. Chem. Soc.*, 1955, **77**, 5118.

already known manner.[66] In the direct formylation of pyrroles, it has been found necessary to use equimolecular amounts of phosphoryl chloride and dimethylformamide; if the proportion of the former is decreased, the yield falls.[67] Reaction presumably proceeds *via* the complex (14) :

$$[Me_2\overset{+}{N}{=}CH{\cdot}O{\cdot}P(O)Cl_2 \longleftrightarrow Me_2\overset{+}{N}{:}CH{\cdot}O{\cdot}P(O)Cl_2]Cl^-$$
(14)

The quaternary salts of tertiary Mannich bases react with phenylhydroxylamine, with amino-group interchange, resulting in *N*-substituted phenylhydroxylamines, which can be readily converted into phenylnitrones and hence hydrolysed to aldehydes.[68] Alternatively the *N* substituted phenylhydroxylamines can be obtained by direct Mannich condensation, with phenylhydroxylamine and formaldehyde. An earlier method for the conversion of gramine into 3-formylindole consisted in treatment with hexamethylenetetramine in dilute acetic acid.[69]

Although esters and acid chlorides react with alkyl- and aryl-lithium compounds to give mainly *tert.*-alcohols, carboxylic acids or their lithium salts often give good yields of ketones. This has now been shown to be due to the formation of a dilithium salt $R_2C(OLi)_2$, which resists further reaction.[70]

A detailed account has been published of the methods used by Reppe and his collaborators to bring about reaction between acetylene and amines or carbonyl compounds; and of the amazing variety of compounds into which the products have been converted.[71]

Selenium dioxide, in the presence of methanol, has been used to prepare a dimethyl ketal from a ketone.[72]

A review of the use of acetoacetaldehyde in organic synthesis [73] and a symposium on " hydroxycarbonylation " [74] have appeared.

The explanation given many years ago [75] for the " abnormal " Michael addition of diethyl methylmalonate to ethyl crotonate has found confirmation through the use of isotopic tracers.[76]

Alcohols.—The trialkylaluminium compounds react with air to form aluminium alkoxides, which are decomposed by water to give *primary* alcohols : [77]

$$2R_3Al + 3O_2 \longrightarrow 2Al(OR)_3$$
$$R'R''C{:}CH_2 \longrightarrow (R'R''CH{\cdot}CH_2)_3Al$$
(R', R'' = H and/or alkyl)

[66] F. Weygand and R. Mitgau, *Chem. Ber.*, 1955, **88**, 301; *Ann. Reports*, 1953, **50**, 167.
[67] R. M. Silverstein, E. R. Ryskiewicz, C. Willard, and R. C. Koehler, *J. Org. Chem.*, 1955, **20**, 668.
[68] J. Thesing, *Chem. Ber.*, 1954, **87**, 507; J. Thesing, A. Müller, and G. Michel, *ibid.*, 1955, **88**, 1027; J. Thesing, H. Uhring, and A. Müller, *Angew. Chem.*, 1955, **67**, 31
[69] H. R. Snyder, S. Swaminathan, and H. J. Sims, *J. Amer. Chem. Soc.*, 1952, **74**, 5110; M. M. Robison and B. L. Robison, *ibid.*, 1955, **77**, 457.
[70] H. F. Bluhm, H. V. Donn, and H. D. Zook, *ibid.*, p. 4406.
[71] W. Reppe, *Annalen*, 1955, **596**, 1—224.
[72] E. P. Oliveto, C. Gerold, and E. B. Hershberg, *J. Amer. Chem. Soc.*, 1954, **76**, 6113.
[73] W. Franke and R. Kraft, *Angew. Chem.*, 1955, **67**, 395.
[74] Various Authors, *Bull. Soc. chim. France*, 1955, 115, 250.
[75] N. E. Holden and A. Lapworth, *J.*, 1931, 2368.
[76] O. Simamura, N. Inamoto, and T. Suehiro, *Bull. Chem. Soc. Japan*, 1954, **27**, 221; O. Simamura and N. Inamoto, *ibid.*, 1955, **28**, 529; G. A. Swan, *J.*, 1955, 1039; D. Samuel and D. Ginsburg, *J.*, 1955, 1288.
[77] K. Ziegler, H. G. Gellert, H. Martin, K. Nagel, and J. Schneider, *Annalen*, 1954, **589**, 91; K. Ziegler, F. Krupp, and K. Zosel, *Angew. Chem.*, 1955, **67**, 425.

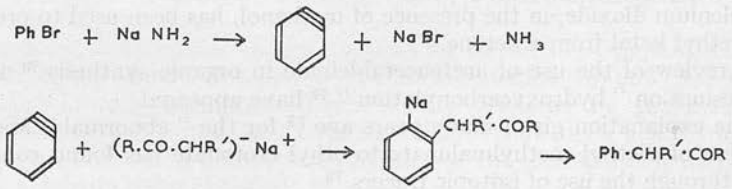
This fills another gap in synthetic methods, as previously the route for the conversion of alkenes into primary alcohols went round *via* the peroxide-catalysed addition of hydrogen bromide.

To provide a sounder basis for the use of 3-nitrophthalic anhydride for the differentiation of primary and secondary alcohols, the reaction rates with a series of alcohols have been measured at 4°. The times found for half reaction were 20—57, 63—450, and 2700—3600 min., respectively, for primary, secondary, and tertiary alcohols.[78]

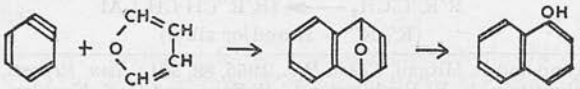
Amides.—An arenesulphonyl halide in the presence of pyridine is recommended for converting sensitive amides into nitriles under mild conditions. Aromatic and αβ-unsaturated amides give high yields.[79]

Although yohimbine failed to yield yohimbamide by conventional methods, the conversion was successfully accomplished by the action of sodamide in liquid ammonia.[80] This method appears worthy of trial in cases of other esters which resist conversion into amides.

" Benzyne " Intermediates.—The recognition of the participation of " benzyne " intermediates in the reactions of aryl halides in the presence of strong bases is leading to the discovery of new reactions.[81] Among these is the direct phenylation of ketones, a process which has not hitherto proved feasible. Thus equimolecular amounts of a sodio-ketone and bromobenzene fail to react; but, if a suspension of sodamide in liquid ammonia is added to this mixture, phenylation ensues : [82]



Another example is the reaction of *o*-bromofluorobenzene with lithium amalgam in the presence of furan, α-naphthol being obtained by hydrolysis of the product with methanolic hydrogen chloride : [83]



Amines.—*N*-Alkylanilines have been obtained from arylamines by refluxing them with primary alcohols, in the presence of Raney nickel,[84] and by treatment with primary alcohols in the presence of the potassium alkoxide

[78] K. Freudenberg and G. Achtzehn, *Chem. Ber.*, 1955, **88**, 10.
[79] C. R. Stephens, E. J. Bianco, and F. J. Pilgrim, *J. Amer. Chem. Soc.*, 1955, **77**, 1701.
[80] C. F. Huebner, R. Lucas, H. B. MacPhillamy, and H. A. Troxell, *ibid.*, p. 469.
[81] G. Wittig, *Naturwiss.*, 1942, **30**, 696; J. D. Roberts, H. E. Simmons, L. A. Carlsmith, and C. W. Vaughan, *J. Amer. Chem. Soc.*, 1953, **75**, 3290; R. Huisgen and H. Rist, *Naturwiss.*, 1954, **41**, 358; A. Lüttringhaus and K. Schubert, *ibid.*, 1955, **42**, 17.
[82] W. W. Leake and R. Levine, *Chem. and Ind.*, 1955, 1160; R. Levine and W. W. Leake, *Science*, 1955, **121**, 780.
[83] G. Wittig and L. Pohmer, *Angew. Chem.*, 1955, **67**, 348.
[84] R. G. Rice and E. J. Kohn, *J. Amer. Chem. Soc.*, 1955, **77**, 4052.

and nickel.[85] Another method consists in heating to 240—250° the condensation product of the amine with a dialkyl phosphochloridate: [86]

$$(RO)_2POCl \longrightarrow (RO)_2PO{\cdot}NHAr \longrightarrow Ar{\cdot}NHR$$

Methyl 2 : 4-dinitrobenzenesulphonate is recommended for the quaternisation of weak organic bases (*e.g.*, phenazine) in cases where this is difficult to effect with methyl sulphate or other reagents.[87] It has been observed that certain alkaloids are converted into dichloromethochlorides merely by being kept at room temperature in chloroform solution.[88]

Miscellaneous.—Ethereal solutions of phenylsodium containing also phenyl-lithium apparently contain $[Ph_2Li]^-Na^+$ and are claimed to be much more stable to storage, yet more reactive towards alkyl halides, etc., than those of phenylsodium itself.[89]

Acetone cyanohydrin nitrate is a reagent which can be used for effecting nitration under alkaline conditions.[90]

In contrast to the poor alkylating properties of phenolic Mannich bases, the sulphur analogue, 1-ethylthiomethyl-2-naphthol alkylates a wide range of compounds smoothly.[91]

The presence of inorganic salts, precipitated with *N*-bromosuccinimide during its preparation, is said to increase the amount of addition accompanying bromination with the reagent.[92]

Other papers deal with the use of silicon tetrachloride to split ether linkages,[93] a new reagent for the optical resolution of aromatic hydrocarbons lacking functional groups,[94] and the preparation of *iso*thiocyanates.[95]

Comprehensive surveys have appeared dealing with, besides other topics, catalytic methods in organic chemistry, methods of optical resolution, and the preparation of large-ring compounds and compounds containing isotopes.[96]

G. A. S.

4. ALIPHATIC COMPOUNDS.

Acetylenes.—The conditions under which acetylene polymerises to *cyclo*octatetraene have been closely defined after a detailed study of all the variable factors.[1] Dioxan is the best solvent, and nickel–acetylacetone the most convenient catalyst; reasonably dry conditions must be maintained but otherwise the reaction is more tolerant than was originally believed.

[85] E. F. Pratt and E. J. Frazza, *J. Amer. Chem. Soc.*, 1954, **76**, 6174.
[86] W. Gerrard and G. J. Jeacocke, *Chem. and Ind.*, 1954, 1538.
[87] A. I. Kiprijanow and A. I. Tolmatschew, 14th Internat. Congr. Pure Appl. Chem., Zürich, 1955, Handbook, p. 320.
[88] M. E. von Klemperer and F. L. Warren, *Chem. and Ind.*, 1955, 1553.
[89] G. Wittig, R. Ludwig, and R. Polster, *Chem. Ber.*, 1955, **88**, 294.
[90] W. D. Emmons and J. P. Freeman, *J. Amer. Chem. Soc.*, 1955, **77**, 4387.
[91] F. Poppelsdorf and S. J. Holt, *J.*, 1954, 4094.
[92] W. J. Bailey and J. Bello, *J. Org. Chem.*, 1955, **20**, 689; E. A. Braude and E. S. Waight, *J.*, 1952, 1116.
[93] R. Schwarz and W. Kuchen, *Angew. Chem.*, 1955, **67**, 347.
[94] M. S. Newman, W. B. Lutz, and D. Lednicer, *J. Amer. Chem. Soc.*, 1955, **77**, 3420.
[95] E. Söderbäck, *Acta Chem. Scand.*, 1954, **8**, 1851; G. J. M. van der Kerk, C. W. Pluyger, and G. de Vries, *Rec. Trav. chim.*, 1955, **74**, 1262.
[96] "Methoden der Organischen Chemie (Houben-Weyl)," G. Thieme, Stuttgart, 4th Edn., 1955, Vol. IV, Pt. 2.
[1] S. N. Ushakov and O. F. Solomon, *Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk*, 1954, 593.

The impressive group of new reactions covered by the term "ethynylation," and numerous transformations of the immediate products, have been reviewed in detail by Reppe.[2]

A study[3] of catalyst specificity has emphasised the superiority of the lead-poisoned palladium catalyst for semihydrogenation,[4] even for use with simple acetylenes.

The number and variety of natural acetylenic compounds continues to grow; the field has been reviewed.[5] The Trondheim school report the isolation[6] from *Correopsis* species of a curious family of linear C_{13} hydrocarbons (1), in which the terminal groups remain constant while the connecting chain varies in unsaturation from $\cdot CH{=}CH\cdot[C{\equiv}C]_2\cdot[CH{=}CH]_2\cdot$, in a hydrocarbon since synthesised,[7] to $\cdot[C{\equiv}C]_5\cdot$, in what must surely be the most highly unsaturated natural product yet discovered. The related acetates (2) and (3), which also have C_{13} main chains, and (probably) the hydro-

$H_2C{=}CH\cdot(C_{10}H_x)\cdot CH_3$ (1)

$H_2C{=}CH\cdot CH{=}CH\cdot[C{\equiv}C]_3\cdot CH{=}CH\cdot CH_2\cdot OAc$ (2)

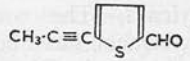
$Ph\cdot[C{\equiv}C]_2\cdot CH{=}CH\cdot CH_2\cdot OAc$ *trans* (3)

$Ph\cdot[C{\equiv}C]_3\cdot[CH{=}CH]_2\cdot CH_3$ (4)

carbon (4) were isolated from the same plants. Compound (3) has already been synthesised.[8]

The ketone isolated from *Artemisia vulgaris*, tentatively formulated as dodeca-4-en-6 : 8 : 10-triyn-2-one,[9] has now been shown to have the C_{14} structure (5) and has been totally synthesised.[10] Some simple polyacetylenes previously found in plants of the Compositæ family, have been isolated[11] from cultures of the Basidiomycete fungus *Polyporus anthracophilus*. The aldehyde (6) is produced by *Dædalea juniperina*, and represents an extension of the existing range of natural acetylenes.[12]

$Me\cdot[C{\equiv}C]_3\cdot CH{=}CH\cdot CH_2\cdot CH_2\cdot COEt$ *trans* (5)



(6)

Synthetical confirmation of structures proposed for the *Oenanthe crocata* and *Cicuta virosa* poly-ynes[13] has now been published.[14, 15] The synthesis[15]

[2] W. Reppe, *Annalen*, 1955, **596**, 1—224.
[3] L. Crombie, *J.*, 1955, 3510.
[4] H. Lindlar, *Helv. Chim. Acta*, 1952, **35**, 446.
[5] F. Bohlmann, *Angew. Chem.*, 1955, **67**, 389.
[6] J. S. Sörensen and N. A. Sörensen, *Acta Chem. Scand.*, 1954, **8**, 1741, 1763; J. S. Sörensen, D. Holme, E. T. Borlaug, and N. A. Sörensen, *ibid.*, p. 1769; E. R. H. Jones, J. M. Thompson, and M. C. Whiting, *ibid.*, p. 1944.
[7] F. Bohlmann and H. J. Mannhardt, *Chem. Ber.*, 1955, **88**, 1330.
[8] T. Bruun, L. Skatteböl, and N. A. Sörensen, *Acta Chem. Scand.*, 1954, **8**, 1757.
[9] K. Stavholt and N. A. Sörensen, *ibid.*, 1950, **4**, 1567, and unpublished work.
[10] F. Bohlmann, H. J. Mannhardt, and H. G. Viehe, *Chem. Ber.*, 1955, **88**, 361.
[11] J. D. Bu'Lock, E. R. H. Jones, and W. B. Turner, *Chem. and Ind.*, 1955, 686.
[12] J. H. Birkinshaw and P. Chaplen, *Biochem. J.*, 1955, **60**, 255.
[13] E. F. L. Anet, B. Lythgoe, M. H. Silk, and S. Trippett, *J.*, 1953, 309.
[14] B. E. Hill, B. Lythgoe, S. Mirvish, and S. Trippett, *J.*, 1955, 1770.
[15] F. Bohlmann and H. G. Viehe, *Chem. Ber.*, 1955, **88**, 1245, 1347.

of cicutol (7) exemplifies the Wittig–Schöllkopf reaction,[16] which will undoubtedly play an increasingly important role :

$$Bu{\cdot}CH{=}CH{\cdot}CH_2Br \xrightarrow{1} Bu{\cdot}CH{=}CH{\cdot}CH_2{\cdot}P^+Ph_3\,Br^- \xrightarrow{2} Bu{\cdot}CH{=}CH{\cdot}CH{=}PPh_3$$

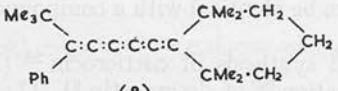
(*trans*)

$$\xrightarrow{3} Bu{\cdot}[CH{=}CH]_3{\cdot}C{\equiv}CH \xrightarrow{4} Bu{\cdot}[CH{=}CH]_3{\cdot}[C{\equiv}C]_2{\cdot}[CH_2]_3{\cdot}OH \quad (7)$$

(mainly all-*trans*; all-*trans*)

Reagents : 1, PPh_3. 2, BuLi. 3, $CH{\equiv}C{\cdot}CH{=}CH{\cdot}CHO$. 4, $CH{\equiv}C{\cdot}[CH_2]_3{\cdot}OH$ ($CuCl$–O_2).

Allenes and Cumulenes.—Phenoxyallene has been reported.[17] A range of cumulenes with only two aromatic substituents has been prepared,[18] and several cumulenes obtained and in some cases isolated,[19] in which bulky end-groups were employed to ensure abnormal stability, *e.g.*, in compound (8).



(8)

Examples include hydrocarbons with one, two, and three phenyl groups, so that the wide gap in stability and in spectroscopic properties between the tetra-arylcumulenes and the purely aliphatic cumulenes described in the preceding Report has been firmly bridged.

Polyenes.—2-Vinylbuta-1 : 3-diene (9), which is perhaps the prototype of all cross-conjugated systems, has been synthesised independently and almost simultaneously by two routes : [20, 21]

$$CMe_2{=}CH_2 + CH_2O + Ac_2O \longrightarrow CH_2{=}CH{\cdot}C(=CH_2){\cdot}CH_2{\cdot}CH_2{\cdot}OAc \xrightarrow{485^\circ}$$

$$CH_2{=}CH{\cdot}C(=CH_2){\cdot}CH{=}CH_2$$

(9)

$$CO_2Et{\cdot}CH_2{\cdot}CH(CO_2Et){\cdot}CH_2{\cdot}CO_2Et \xrightarrow{1} CH_2(OAc){\cdot}CH_2{\cdot}CH(CH_2{\cdot}OAc){\cdot}CH_2{\cdot}CH_2{\cdot}OAc$$

(540°)

Reagent : 1, $LiAlH_4$–Ac_2O.

Unfortunately there are large differences in the physical constants and spectroscopic properties recorded for the two products (even if the ε value [21] of 205,000 is a misprint for 20,500), and it would seem that one at least must have been very impure.

$$CH_3{\cdot}[CH{=}CH]_a{\cdot}CHO + C_2H_2 + CHO{\cdot}[CH{=}CH]_b{\cdot}CH_3 \xrightarrow{1}$$

$$CH_3{\cdot}[CH{=}CH]_a{\cdot}CH(OH){\cdot}C{\equiv}C{\cdot}CH(OH){\cdot}[CH{=}CH]_b{\cdot}CH_3 \xrightarrow{2} CH_3{\cdot}[CH{=}CH]_n{\cdot}CH_3$$

($n = a + b + 2$) (10)

Reagents : 1, 2 stages. 2, $LiAlH_4$.

The chemistry of hexa-1 : 3 : 5-triene has been investigated.[22] A series of αω-dimethylpolyenes (10; $n = 3$—10 and 12) has been prepared [23] with

[16] G. Wittig and U. Schöllkopf, *Chem. Ber.*, 1954, **87**, 1318.
[17] L. F. Hatch and H. D. Weiss, *J. Amer. Chem. Soc.*, 1955, **77**, 1798.
[18] R. Kuhn and H. Krauch, *Chem. Ber.*, 1955, **88**, 309.
[19] F. Bohlmann and K. Kieslich, *ibid.*, p. 1211.
[20] A. T. Blomquist and J. A. Verdol, *J. Amer. Chem. Soc.*, 1955, **77**, 81.
[21] W. J. Bailey and J. Economy, *ibid.*, p. 1133.
[22] G. F. Woods, N. C. Bolgiano, and D. E. Duggen, *ibid.*, p. 1800.
[23] P. Nayler and M. C. Whiting, *J.*, 1955, 3037.

the object of testing various theories of electronic spectra, but the results were only in qualitative agreement with these.

Interest in the geometrical isomerism of conjugated systems is still evident. The two possible bis-2 : 2′-diphenylylenehexa-1 : 3 : 5-trienes, the two phenyl bis-2 : 2′-diphenylylenebutadienes, and the three phenyl bis-2 : 2′-diphenylylenehexatrienes have all been isolated.[24] Some much simpler dienes have recently been obtained in all possible forms; these include the four deca-2 : 4-dienoic acids, esters, and amides,[25] the four deca-2 : 4-dien-1-ols,[25] the hexa-2 : 4-dienoic acids and esters,[26] and the hexa-2 : 4-dien-1-ols.[27] The two groups who investigated the C_6 compounds were able to work entirely with crystallisable compounds which could be rigorously purified. The four $\Delta^{2:4}$-hexenynoic acids and esters were also prepared. Spectroscopic data were recorded by all three groups. Deca-*trans*-2 : *cis*-4-dienoic acid proved [25] to be identical with a compound isolated from *Sapium sebiferum* Roxb.[28]

Details of a second synthesis of corticrocin [29] (11; $n = 6$) have now appeared, and the isolation [30] of descrocetin [31] (11; $n = 7$) by hydrolysis of the antibiotic limocrocin has been described.

Remarkable progress has been made on the geometrical isomerism of vitamin A_1 and retinene$_1$. Five isomers have been obtained pure from each,[32] and their configurations have been established as a result of stereospecific syntheses, spectroscopic studies, and determination of biological activity.[33]

$HO_2C{\cdot}[CH{=}CH]_n{\cdot}CO_2H$ (11)

$R_\beta{\cdot}CH{:}CH{\cdot}CMe{:}CH{\cdot}CH{:}CH{\cdot}CMe{:}CH{\cdot}CH{:}NMe$ (13)

$X{:}CH{\cdot}CH{:}CMe{\cdot}CH{:}CH{\cdot}CH{:}CMe{\cdot}CH{:}CH_2$ (12)

(X = 4-ethoxy-2 : 6 : 6-trimethyl*cyclo*hex-2-enylidene)
(R_β = 2 : 6 : 6-trimethyl*cyclo*hex-1-enyl)

Vitamin A_2 has been prepared directly from vitamin A_1 by oxidation of the latter to retinene$_1$, allylic bromination, dehydrobromination, and reduction.[34] Its "anhydro-derivative" has been shown [35] to be ethoxyanhydrovitamin A_1 (12).

Methylretinylideneamine$_1$ (13) reproduces accurately the spectroscopic behaviour of "indicator yellow." [36]

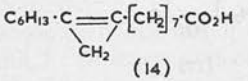
[24] E. F. Magoon and L. Zechmeister, *J. Amer. Chem. Soc.*, 1955, **77**, 5642.
[25] L. Crombie, *J.*, 1955, 1007.
[26] J. L. H. Allan, E. R. H. Jones, and M. C. Whiting, *J.*, 1955, 1862; J. L. H. Allan, G. D. Meakins, and M. C. Whiting, *J.*, 1955, 1874.
[27] A. Butenandt, E. Hecker, and H. G. Zachau, *Chem. Ber.*, 1955, **88**, 1185.
[28] A. Crossley and T. P. Hilditch, *J.*, 1949, 3353.
[29] B. C. L. Weedon, *J.*, 1954, 4168.
[30] H. Brockmann and H. U. May, *Chem. Ber.*, 1955, **88**, 419.
[31] R. Kuhn and C. Grundmann, *Ber.*, 1937, **70**, 1318.
[32] C. D. Robeson, J. D. Cawley, L. Weisler, M. H. Stern, C. C. Eddinger, and A. J. Chechak, *J. Amer. Chem. Soc.*, 1955, **77**, 4111; C. D. Robeson, W. P. Blum, J. M. Dieterle, J. D. Cawley, and J. G. Baxter, *ibid.*, p. 4120; J. D. Cawley, *ibid.*, p. 4125; J. D. Cawley and D. R. Nelan, *ibid.*, p. 4130.
[33] S. R. Ames, W. J. Swanson, and P. L. Harris, *ibid.*, p. 4136.
[34] H. B. Henbest, E. R. H. Jones, and T. C. Owen, *J.*, 1955, 2765.
[35] H. B. Henbest, E. R. H. Jones, T. C. Owen, and V. Thaller, *J.*, 1955, 2763.
[36] G. A. Pitt, F. D. Collins, R. A. Morton, and P. Stok, *Biochem. J.*, 1955, **59**, 122.

Some progress in the study of the *cis*-isomers of carotenoids has resulted from infrared investigations,[37] which have also revealed an allenic linkage in fucoxanthin.[38] The use of adsorption chromatography on derived *p*-phenylazoanils permits isolation of the αα-dimethyl-glutaric and -succinic acids formed in small quantity by oxidative degradation of carotenoids and related compounds,[39] while the application of paper chromatography [38] should provide even greater sensitivity.

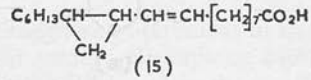
The 10 : 11-mono-*cis*-isomer of methylbixin has been synthesised, and the properties of a number of stereoisomers have been compared.[40]

Lipids.—The αω-dihydroxy-*n*-paraffins from C_{22} to C_{28} (even numbers), and the corresponding hydroxy-acids from C_{18} to C_{36}, have been isolated from carnauba wax.[41]

The constitution (14) proposed for sterculic acid [42] has been challenged [43]

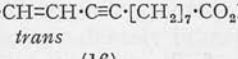


(14)

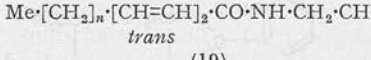


(15)

and defended.[44] Discussion must be deferred until full details have been published of the synthesis [43] from diazomethane and stearolic acid of a product said to be the acid (14) and to differ from sterculic acid, and of the Indian workers' explanation of the formation, on ozonolysis, of 9 : 11-dioxononadecanoic acid in terms of their preferred structure (15) for sterculic acid. A C_{18} analogue of this remarkable acid is present in kapok seed oil.[44] Further confirmatory work on ximenynic (santalbic) acid (16) has been described.[45]



(16)



(19)

Methods of improving the anodic coupling method for the synthesis of fatty acids have been discussed,[46] and their application to unsaturated acids critically examined.[47] The heterogeneity of "vaccenic acid" has been confirmed.[48] One of the syntheses of ricinoleic acid reported last year has been fully described,[49] and brief accounts have appeared of syntheses, by routes involving acetylenic intermediates, of linolenic [50] and α-elæostearic [51]

[37] K. Lunde and L. Zechmeister, *J. Amer. Chem. Soc.*, 1955, **77**, 1647.
[38] F. G. Torto, N. F. Holyer and B. C. L. Weedon, *Chem. and Ind.*, 1955, 1219.
[39] H. B. Henbest and T. C. Owen, *J.*, 1955, 2968.
[40] H. H. Inhoffen and G. Raspé, *Annalen*, 1955, **592**, 214.
[41] K. E. Murray and R. Schoenfeld, *Austral. J. Chem.*, 1955, **8**, 432, 437.
[42] J. R. Nunn, *J.*, 1952, 313.
[43] J. P. Verma, B. Nath, and J. S. Aggarwal, *Nature*, 1955, **175**, 84; **176**, 1082.
[44] G. Dijkstra and H. J. Duin, *ibid.*, p. 71.
[45] J. Grigor, D. M. MacInnes, J. McLean, and A. J. P. Hogg, *J.*, 1955, 1069; F. D. Gunstone and W. C. Russell, *J.*, 1955, 3782.
[46] R. P. Linstead, B. C. L. Weedon, and B. Wladislaw, *J.*, 1955, 1097.
[47] B. W. Baker, R. P. Linstead, and B. C. L. Weedon, *J.*, 1955, 2218.
[48] D. G. Bounds, R. P. Linstead, and B. C. L. Weedon, *J.*, 1954, 4219.
[49] L. Crombie and A. G. Jacklin, *J.*, 1955, 1740.
[50] S. S. Nigam and B. C. L. Weedon, *Chem. and Ind.*, 1955, 1555.
[51] L. Crombie and A. G. Jacklin, *ibid.*, p. 1187.

acids, that of the latter finally establishing the configuration of the conjugated system :

$$C_4H_9\cdot CH{=}CH\cdot CHO \xrightarrow{1} C_4H_9\cdot CH{=}CH\cdot CH(OH)\cdot CH_2\cdot C{\equiv}CH \xrightarrow{2}$$
trans

$$C_4H_9\cdot[CH{=}CH]_2\cdot C{\equiv}CH \xrightarrow{3} C_4H_9\cdot[CH{=}CH]_2\cdot C{\equiv}C\cdot[CH_2]_7\cdot Cl \xrightarrow{4}$$

$$C_4H_9\cdot[CH{=}CH]_2\cdot C{\equiv}C\cdot[CH_2]_7\cdot CO_2H \xrightarrow{5} C_4H_9\cdot CH{=}CH\cdot CH{=}CH\cdot CH{=}CH\cdot[CH_2]_7\cdot CO_2H$$
trans *trans* *trans* *cis*

Reagents : 1, $HC{\equiv}C\cdot CH_2Br$–Zn. 2, PCl_3–KOH. 3, $I\cdot[CH_2]_7\cdot Cl$–$NaNH_2$. 4, NaI, NaCN–KOH. 6, H_2.

The synthesis of (±)-lichesterinic acid (17),[52] a transformation product of the natural acid (18), confirms the structure of the latter.

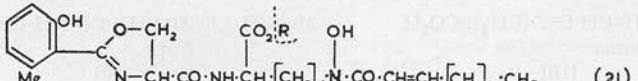
(18) (17)

Further details have appeared of work on affinin,[53] anacyclin,[54] and *neo*herculin.[55] The substance long known as "pellitorine" is heterogeneous, with the amides (19; $n = 4$, 6, and 8) as the main constituents.[54]

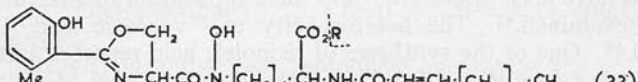
Phthiocerol is now believed to be the 1 : 3-glycol (20).[56] The bacterial

$$CH_3\cdot[CH_2]_{22}\cdot CH(OH)\cdot CH_2\cdot CH(OH)\cdot C_9H_{18}\cdot OMe$$
(20)

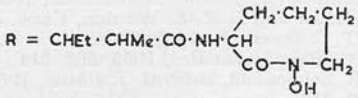
growth factor mycobactin is probably either (21) or (22); [57] it is thus one of the most complex molecules whose structures have yet been unravelled.



(21)



(22)



Cleavage giving *m*-cresol, carbon dioxide, serine, (—)-3-hydroxy-2-methylpentanoic acid, octadec-2-enoic acid, and two molecules of *N*-hydroxylysine

[52] E. E. van Tamelen, C. E. Osborne, and S. R. Bach, *J. Amer. Chem. Soc.*, 1955, **77**, 4625.
[53] M. Jacobson, *ibid.*, p. 2461.
[54] L. Crombie, *J.*, 1955, 999.
[55] *Idem*, *J.*, 1955, 995.
[56] J. A. Hall, J. W. Lewis, and N. Polgar, *J.*, 1955, 3971.
[57] G. A. Snow, *J.*, 1954, 4080.

was followed by partial hydrolysis as indicated. This permitted recognition through ultraviolet absorption spectra of the *o*-hydroxyphenyloxazoline and hydroxamic acid groupings in the main (" mycobactic acid ") fraction, and of the cyclic hydroxamic acid system in the smaller (" cobactin ") fragment through pK_a measurements and further degradation. In either case methylation and hydrolysis gave *N*-methoxylysine.

Miscellaneous.—A group of *cis*-αβ-ethylenic carbonyl compounds (dec-3-en-2-one, hept-3-en-2-one,[58] benzylideneacetone and cinnamaldehyde) [59] has been prepared by semihydrogenation of the corresponding acetylenes. They are fairly stable, at least under neutral or alkaline conditions. The *cis*-hept-3-en-2-one differed from the substance previously [60] assigned this structure, which has been shown [61] to be a mixture of the *trans*-isomer and 2-ethylhex-2-en-1-al. 3-Ethylpent-3-en-2-one, with a trisubstituted ethylenic linkage, has also been resolved into its two geometrical isomers.[62]

Brief accounts of the use of alkenylmagnesium bromides in the synthesis of 1 : 3- and 1 : 4-dienes, and of a great variety of ethylenic alcohols, have appeared.[63] In ease of formation, in versatility, and in the excellent yields of products obtained the alkenylmagnesium bromides appear to be analogous to saturated or aromatic Grignard reagents, and it is indeed astonishing that their utilisation has been so long delayed. It is to be hoped that full details will be published soon, especially since failure to effect one of these reactions has been reported.[64] Alkenyl-lithium compounds react smoothly with dimethylformamide to give αβ-ethylenic aldehydes.[65]

Diazomethane is now known to possess pseudo-acidic properties, giving a lithium derivative which converts, *e.g.*, benzaldehyde into 2-phenyloxadiazole.[66] The lithium derivative, and hence diazomethane itself, is obtained when nitrous oxide is added to methyl-lithium.[67]

Some very densely branched compounds have recently been prepared,[68] and their properties are in many ways remarkable. Thus the *p*-nitrobenzoate (27) undergoes solvolysis readily, giving as main product an olefin (28) in which two Wagner–Meerwein rearrangements have taken place.[69]

$$(CMe_3)_2CO + LiCMe_3 \longrightarrow (CMe_3)_3C{\cdot}OLi \longrightarrow \underset{(27)}{(CMe_3)_3C{\cdot}O_2C{\cdot}C_6H_4{\cdot}NO_2}$$

$$\longrightarrow [(CMe_3)_3C^+] \longrightarrow [{}^+CMe_2{\cdot}CMe(CMe_3)_2] \longrightarrow$$

$$[CMe_3{\cdot}CMe_2{\cdot}C^+Me{\cdot}CMe_3] \longrightarrow \underset{(28)}{CMe_3{\cdot}CMe_2{\cdot}C(:CH_2){\cdot}CMe_3}$$

Pyrolysis of secondary acetates at *ca.* 500° gives, as almost sole product, the least alkylated olefin.[70] In this it differs from the milder Tschugaeff

[58] V. Theus, W. Surber, L. Colombi, and H. Schinz, *Helv. Chim. Acta*, 1955, **38**, 239.
[59] G. Gamboni, V. Theus, and H. Schinz, *ibid.*, p. 255.
[60] E. N. Eccot and R. P. Linstead, *J.*, 1930, 905.
[61] G. de Gaudemaris and P. Arnaud, *Compt. rend.*, 1955, **241**, 1311.
[62] R. Heilmann, G. de Gaudemaris, and P. Arnaud, *ibid.*, 1955, **240**, 1433.
[63] H. Normant, *ibid.*, 1954, **239**, 1811; 1955, **240**, 314, 440, 631, 1111, 1435.
[64] E. A. Braude and E. A. Evans, *J.*, 1955, 3324.
[65] *Idem*, *J.*, 1955, 3334.
[66] E. Müller and D. Ludsteck, *Chem. Ber.*, 1954, **87**, 1887; 1955, **88**, 921.
[67] E. Müller, D. Ludsteck, and W. Rundel, *Angew. Chem.*, 1955, **67**, 617.
[68] P. D. Bartlett and E. B. Lefferts, *J. Amer. Chem. Soc.*, 1955, **77**, 2804.
[69] P. D. Bartlett and M. Stiles, *ibid.*, p. 2806.
[70] W. J. Bailey and C. King, *ibid.*, p. 75.

reaction, and it should thus be valuable in synthetical work; mechanistically, however, the result is puzzling.

Hydration of olefins, with a directional specificity opposite to that of the acid-catalysed reaction, is possible by the use of triethylaluminium followed by oxygen.[71]

A group of *iso*thiocyanates is present, as glucosides, in plants of the Cruciferæ family. In addition to the long-known "mustard oils" the methyl, 4-methylthiobutyl, and 3-methylthiopropyl *iso*thiocyanates are now known [72] to occur naturally.

M. C. W.

5. AROMATIC COMPOUNDS.

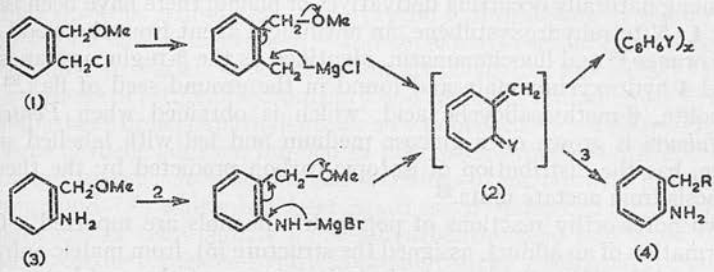
Sixty-nine aromatic hydrocarbons, mainly alkylbenzenes, have been characterised by *C*-acetylation and formation of carbonyl derivatives of the resultant acetophenones.[1] Metallic potassium and sodium oxide provide a highly selective combination for metalating alkylarylhydrocarbons in the α-position of the side-chain.[2] Ethylation at the same position may be effected with ethylene under pressure and a catalyst of sodium and an organic promoter such as anthracene.[3] The same type of catalyst is used to isomerise olefinic hydrocarbons: (+)-limonene is thereby isomerised to conjugated menthadienes and also dehydrogenated to *p*-cymene.[4] Lithium in anhydrous ethylamine is recommended as a selective reagent for reducing aromatic hydrocarbons to cyclic mono-olefins. Thus, in one operation naphthalene or tetralin yields $\Delta^{9(10)}$-octalin and some of the $\Delta^{1(9)}$-isomer, benzene yields *cyclo*hexene and *cyclo*hexane, and ethylbenzene yields 1-ethyl-*cyclo*hexene alone (at −78°) or with ethyl*cyclo*hexane (at 17°).[5] Rhodium has been used as catalyst for hydrogenating a number of aryl to *cyclo*hexyl compounds.[6]

Bromination of di-*p*-nitrophenylmethane in concentrated sulphuric acid and in presence of silver ions affords [7] a high yield of di-(2-bromo-4-nitrophenyl)methane, whereas the usual methods give di-*p*-nitrophenylmethyl bromide. With 2-hydroxy-acetophenone and -propiophenone, as with their *p*-isomers, side-chain bromination predominates in anhydrous acetic acid and nuclear bromination in aqueous acetic acid.[8] An uncommon reorientation occurs through the action of hot concentrated sulphuric acid on 2 : 3-dinitro-aniline or -acetanilide whereby quantities of 2 : 5- and 3 : 4-dinitro-anilines are formed: 2 : 3-dinitrophenol is likewise partially converted into 2 : 5-dinitrophenol.[9]

As vinylogues of 2-alkyloxyethyl chloride, *o*- and *p*-alkyloxymethylbenzyl

[71] K. Ziegler *et al.*, *Angew. Chem.*, 1955, **67**, 424 et seq.
[72] A. Kjaer and R. Gmelin, *Acta Chem. Scand.*, 1955, **9**, 542; A. Kjaer, R. Gmelin, and I. Larsen, *ibid.*, pp. 857, 1143.
[1] H. Pines and A. W. Shaw, *J. Org. Chem.*, 1955, **20**, 373.
[2] C. E. Claff and A. V. Morton, *ibid.*, p. 981.
[3] H. Pines, J. A. Veseley, and V. N. Ipatieff, *J. Amer. Chem. Soc.*, 1955, **77**, 554.
[4] *Idem*, *ibid.*, p. 347.
[5] R. A. Benkeser, R. E. Robinson, D. M. Sauve, and O. H. Thomas, *ibid.*, p. 3230.
[6] L. D. Freedman, G. O. Doak, and E. L. Petit, *ibid.*, p. 4262.
[7] J. H. Gorvin, *J.*, 1955, 83.
[8] Ng. Ph. Buu-Hoï and D. Lavit, *J.*, 1955, 18.
[9] K. H. Pausacker and J. G. Scroggie, *J.*, 1955, 1897.

chlorides, *e.g.* (1), react with magnesium forming polymers of *o*- and *p*-quinodimethane (2; Y = CH_2).[10] The comparable reaction between *o*- or *p*-methoxymethylanilines, *e.g.* (3), and a Grignard reagent yields the *o*- or *p*-alkylaniline, *e.g.* (4), as main product with varying quantities of polymer from the intermediate (2; Y = NH).[11] These reactions afford a route to otherwise inaccessible alkyl- or arylalkyl-anilines. *N*-Phenyl-*o*-phenylenediamines may be prepared by the action of phenyl-lithium on benzo-2 : 1 : 3-thiadiazoles or their selenium analogues.[12]



Reagents : 1, Mg. 2, RMgBr. 3, (*a*) RMgBr, (*b*) H^+.

A second monotropic polymorph of azoxybenzene has been discovered and the kinetics of its transformation into the stable form studied.[13] The absorption spectra of *o*-carboxyazobenzenes indicate that internal hydrogen bonding contributes to the fine-structure of the molecules.[14] Irradiation of corresponding *trans*-compounds leads to the *cis*-1 : 1′-, *cis*-2 : 2′-, and *cis*-1 : 2′-azonaphthalenes.[15] In representative cases, three of the four possible azoxy-compounds, Ar·CH_2·N_2O·Ar′, have been prepared and assigned *cis*- or *trans*-structures.[16] Contrary to views previously held the "oxide" obtained by peroxy-acid oxidation of the hydrazone, Ar·CH:N·NHAr′, is the *cis*-benzylazoxybenzene, Ar·CH_2·N:N(O)·Ar′. The two *trans*-isomers are simultaneously formed by similar oxidation of the azo-compound, Ar·CH_2·N:NAr′.

Diaryliodonium salts, for which improved methods of preparation are now available,[17] promise to supply a long-felt need for a new arylating agent. Thus, under relatively mild conditions diphenyliodonium bromide phenylates alcohols, thiols, phenols, and amines among other compounds,[18] and the kinetics of its reaction with the phenoxide ion have been examined.[19] Bromobenzene in liquid ammonia and in presence of sodamide phenylates

[10] F. G. Mann and F. H. C. Stewart, *J.*, 1954, 2826.
[11] *Idem, ibid.*, p. 4127.
[12] E. S. Lane and C. Williams, *J.*, 1955, 1468.
[13] A. Hodkin and D. Taylor, *J.*, 1955, 489.
[14] W. C. J. Ross and G. P. Warwick, *Chem. and Ind.*, 1955, 745.
[15] M. Frankel, R. Wolovsky, and E. Fischer, *J.*, 1955, 3441.
[16] J. N. Brough, B. Lythgoe, and (in part) P. Waterhouse, *J.*, 1954, 4069.
[17] F. M. Beringer, M. Drexler, E. M. Gindler, and C. C. Lumpkin, *J. Amer. Chem. Soc.*, 1953, **75**, 2705.
[18] F. M. Beringer, A. Brierley, M. Drexler, E. M. Gindler, and C. C. Lumpkin, *ibid.*, p. 2708.
[19] E. S. Lewis and C. A. Stout, *ibid.*, 1954, **76**, 4619; F. M. Beringer and E. M. Gindler, *ibid.*, 1955, **77**, 3203.

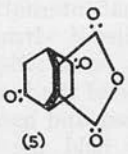
the sodio-derivatives of alkyl ketones: for instance, ethyl α-methylbenzyl ketone is obtained in 60% yield from diethyl ketone.[20]

Monohydric phenols can be converted into hydrocarbons through reduction of aryl diethyl phosphates by sodium or lithium in liquid ammonia.[21] The intermediate esters, which need not be purified, are prepared by adding triethylamine to a solution of the phenol and diethyl hydrogen phosphite in carbon tetrachloride:

$$ArOH + HO{\cdot}P(OEt)_2 + CCl_4 + NEt_3 \longrightarrow ArO{\cdot}P(O)(OEt)_2 + CHCl_3 + NHEt_3Cl$$

Among naturally occurring derivatives of phenol there have been isolated 2 : 3′ : 4 : 5′-tetrahydroxystilbene, an antifungal agent from the wood of the Osage orange,[22] and linocinnamarin, identified as the β-D-glucopyranoside of methyl 4-hydroxycinnamate and found in the ground seed of flax.[23] The metabolite, 6-methylsalicyclic acid, which is obtained when *Penicillium griseofulvum* is grown on a glucose medium and fed with labelled sodium acetate, has the distribution of isotopic carbon predicted by the theory of biogenesis from acetate units.[24]

Two noteworthy reactions of polyhydric phenols are reported. One is the formation of an adduct, assigned the structure (5), from maleic anhydride and quinol.[25] The other is a hydrolytic scission of the *cyclo*hexenylpyrogallol trimethyl ether (6) to pyrogallol and *cyclo*hexanone by the action of demethylating agents: analogously constituted catechol dimethyl ethers are similarly affected.[26]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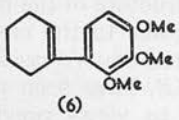

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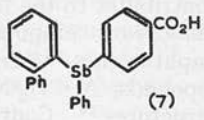


(7)

Dinitrogen tetroxide in carbon tetrachloride or chloroform is the reagent of choice for oxidising benzyl alcohols to benzaldehydes and, coupled with reduction of benzoic esters to prepare the alcohols, this provides a valuable general route to the aldehydes.[27] An alternative method combines Friedel-Crafts formation of aroyl-*N*-methylanilides[28] with the known subsequent reduction of these compounds to arylaldehydes by lithium aluminium hydride:

$$ArH + Cl{\cdot}CO{\cdot}NMePh \longrightarrow Ar{\cdot}CO{\cdot}NMePh \longrightarrow Ar{\cdot}CHO$$

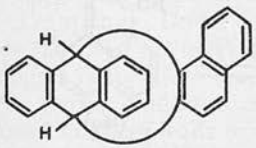
Phthalaldehydic acids may be prepared from phthalides by treatment with dimethylamine, followed by oxidation and hydrolysis of the resultant amides.[29] Successful use has been made of partition chromatography for

[20] W. W. Leake and R. Levine, *Chem. and Ind.*, 1955, 1160.
[21] G. W. Kenner and N. R. Williams, *J.*, 1955, 522.
[22] R. A. Barnes and N. N. Gerber, *J. Amer. Chem. Soc.*, 1955, **77**, 3259.
[23] H. J. Klosterman, F. Smith, and C. O. Clagett, *ibid.*, p. 420.
[24] A. J. Birch, R. A. Massy-Westropp, and C. J. Moye, *Chem. and Ind.*, 1955, 683.
[25] R. C. Cookson and N. S. Wariyar, *ibid.*, p. 915.
[26] J. M. Bruce and F. K. Sutcliffe, *ibid.*, p. 745.
[27] B. O. Field and J. Grundy, *J.*, 1955, 1110.
[28] F. Weygand and R. Mitgau, *Chem. Ber.*, 1955, **88**, 301.
[29] J. Blair, J. J. Brown, and G. T. Newbold, *J.*, 1955, 708.

separating mixed aromatic acids [30] and the syntheses of the 3-methoxy- and 3-hydroxy-derivatives of benzene-1 : 2 : 5-tricarboxylic acid and of benzene-1 : 2 : 4 : 5-tetracarboxylic acid provide useful reference compounds for degradative work.[31]

The optical stability found in enantiomorphs of the stibine (7) supports the view that a pyramidal structure is more firmly retained in tervalent antimony than in tervalent nitrogen compounds.[32]

Polycyclic Compounds.—In this field synthetical activity is distributed between a search for new methods and the adaptation of established ones to particular projects. Acylation of polycyclic hydrocarbons by the Friedel–Crafts reaction has been reviewed [33] and cyclodehydrogenation of large-ring *cyclo*paraffins, to varied types of aromatic polycycle, reported.[34] Details of the synthesis of zethrene [35] from chrysene and of its formation by pyrolysis of acenaphthene or acenaphthylene are now published.[36] Of general interest is the type of diene addition implicit in the condensation of anthracene with 1-nitronaphthalene : it is accompanied by loss of nitrous acid and leads to the compound (8) which has been synthesised independently.[37] In the *meso*-positions of (non-planar) 9 : 10-dihydroanthracene two types of geometrically distinct carbon–hydrogen bond can be distinguished and the conformation in *cis*- and *trans*-9 : 10-disubstituted compounds provides a basis for interpreting their varied reactivity.[38] The well-known ability of 2 : 4 : 5 : 7-tetranitrofluorenone to form molecular complexes is retained in the optically active derivative (9) made by condensing the fluorenone with



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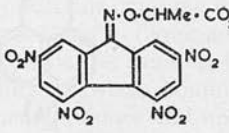

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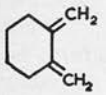

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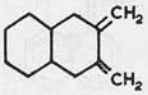

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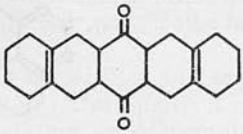


(14)

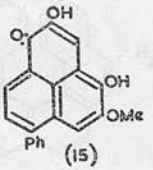
(−)- or (+)-α-(*iso*propylideneaminoxy)propionic acid. Through the complex formed with this reagent the phenanthrophenanthrene (10) has been resolved and becomes the first known optically active hydrocarbon which

[30] P. M. Bhargava and C. Heidelberger, *J. Amer. Chem. Soc.*, 1955, **77**, 166.
[31] J. C. Roberts, *J.*, 1955, 2989.
[32] I. G. M. Campbell, *J.*, 1955, 3116; cf. *Ann. Reports*, 1954, **51**, 159.
[33] P. H. Gore, *Chem. Rev.*, 1955, **55**, 229.
[34] V. Prelog, V. Boarland, and S. Polyák, *Helv. Chim. Acta*, 1955, **38**, 434.
[35] *Ann. Reports*, 1953, **50**, 191.
[36] E. Clar, K. F. Lang, and H. Schulz-Kiesow, *Chem. Ber.*, 1955, **88**, 1520.
[37] C. D. Hurd and L. H. Juel, *J. Amer. Chem. Soc.*, 1955, **77**, 601.
[38] A. H. Beckett and B. A. Mulley, *Chem. and Ind.*, 1955, 146.

owes its asymmetry to molecular overcrowding.[39] The specific rotation, $[\alpha]_D^{24}$ $-3640°$, is in good accord with that calculated on Kirkwood's polarisability theory.[40]

W. J. Bailey's synthesis of pentacene [41] has now been extended to other linear hydrocarbons.[42] It consists essentially of an addition of the diene (11) or of the derived analogue (12) [43] to benzoquinone (13), followed by conversion of the adduct into the fully aromatic compound. Thereby components (11) and (13) *via* the adduct (14) lead to pentacene; (12) and (13) similarly yield heptacene; while (11) and (12) *via* the unsymmetrical adduct with the quinone (13) yield hexacene. Attempted preparation of 4 : 5-dimethylene-*cyclo*hexene fails through spontaneous change to *o*-xylene.[44]

The naturally-occurring glycoside, hæmocorin, affords an unusual type of aglycone for which formula (15) represents one of several possible structures based on 2-hydroxyperinaphthen-1-one.[45] This accommodates the spectroscopic and degradative evidence, the presence of an enolised α-dicarbonyl group, and also *O*-methylation to a pair of isomeric monomethyl and to *two* corresponding dimethyl derivatives. The tautomeric potentialities of the structure are confirmed in simpler cases such as the compound (16 or 16*a*) which likewise affords two methyl ethers identified by oxidation to 2- and 4-methoxynaphthalic anhydride, respectively.[45] On the other hand 9-hydroxyperinaphthen-1-one (17) resists methylation. The compounds (16 or 16*a*) and (17) may be prepared by rearrangement and dehydrogenation from 3 : 4-dihydro-5 : 6-benzocoumarin and 5 : 6-benzochromanone, respectively.[46] Polarographic examination [47] confirms the



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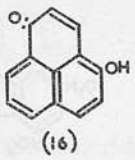

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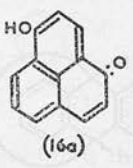

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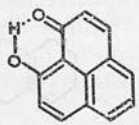

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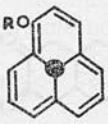
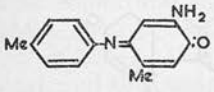


(18)

ease with which the parent perinaphthenone (16; H for OH) is reduced to the hydroxyperinaphthyl radical (18; R = H) which corresponds to the methyl and acetyl derivatives (18; R = Me or Ac) described by Clar.[48]

[39] M. S. Newman, W. B. Lutz, and D. Lednicer, *J. Amer. Chem. Soc.*, 1955, **77**, 3420.
[40] D. D. Fitts and J. G. Kirkwood, *ibid.*, p. 4940.
[41] *Ann. Reports*, 1953, **50**, 190.
[42] W. J. Bailey and C. Liao, *J. Amer. Chem. Soc.*, 1955, **77**, 992.
[43] *Idem*, with G. H. Coleman, *ibid.*, p. 990.
[44] J. E. Ladbury and E. E. Turner, *J.*, 1954, 3885, but cf. ref. 5 in section of Alicyclic Compounds.
[45] R. G. Cooke and W. Segal, *Austral. J. Chem.*, 1955, **8**, 105, 413.
[46] J. D. Loudon and R. K. Razdan, *J.*, 1954, 4299.
[47] P. Beckmann and H. Silberman, *Chem. and Ind.*, 1955, 1635.
[48] E. Clar, "Aromatische Kohlenwasserstoffe," Springer, Berlin, 1952.

Quinones.—2 : 5-Dimethoxybenzoquinone now joins methoxy- and 2 : 6-dimethoxy-benzo-1 : 4-quinone as a natural product : it was isolated from the culture medium of *Polyporus fumosus*.[49] *p*-Substituted monohydric phenols are oxidised by potassium nitrosodisulphonate (Fremy's salt) to *o*-benzoquinones,[50] and the infrared spectra of a number of these products are recorded.[51] The same reagent oxidises primary or secondary arylamines, which have a free *o*- or *p*-position, to *o*- or *p*-quinoneanils, the anil (19) being obtained from *p*-toluidine.[52]



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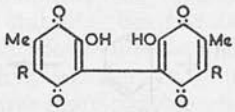

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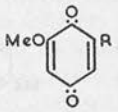

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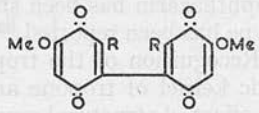

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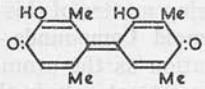


(23)

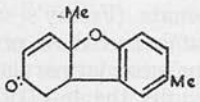
Oosoporein (20; R = OH), a metabolite of the mould *Oosopora colorans*,[53] is also recognised as a metabolite of *Chaetomium aureum* Chivers.[54] In it, as in phoenicin (20; R = H) from *Penicillium* spp., the symmetrical structure suggests an origin from the appropriate monocyclic quinone by some form of coupling. Under acid conditions coupling does occur with quinones of type (21; R = H or Pr^n) but it leads to diquinones of type (22), and the mechanism is therefore unlikely to be the same as that of the natural process.[55] Aerial oxidative coupling between homologues of catechol and resorcinol in alkali leads to diphenoquinones and underlies the formation of red colouring matters from the phenolic concentrates of coal tar.[56] In particular the quinone (23), which can have three tautomeric forms, is almost quantitatively formed from 3 : 5-dimethylcatechol and 2 : 4-dimethylresorcinol.

The crystalline dimer, formed by oxidation of *p*-cresol and originally formulated as (24),[57] is now reformulated as (25). This affords a rational explanation of the oxidation mechanism, is confirmed experimentally, and provides the basis of an elegant synthesis of (±)-usnic acid through dehydration of the corresponding dimer (26) which is obtained by oxidising methylphloracetophenone.[58]

Furanonaphthaquinones are found among the oxidation products of

[49] J. D. Bu'Lock, *J.*, 1955, 575.
[50] H. J. Teuber and G. Staiger, *Chem. Ber.*, 1955, **88**, 802.
[51] W. Otting and G. Staiger, *ibid.*, p. 828.
[52] L. Horner and K. Sturm, *ibid.*, p. 329.
[53] F. Kogl and G. C. van Wessem, *Rec. Trav. chim.*, 1944, **63**, 5.
[54] G. Lloyd, A. Robertson, G. B. Sankey, and W. B. Whalley, *J.*, 1955, 2163.
[55] F. M. Dean, A. M. Osman, and A. Robertson, *J.*, 1955, 11.
[56] W. Baker and D. Miles, *J.*, 1955, 2089.
[57] R. Pummerer, H. Puttfarcken, and P. Schopflocker, *Ber.*, 1925, **58**, 1808.
[58] D. H. R. Barton, A. M. Deflorin, and O. E. Edwards, *Chem. and Ind.*, 1955, 1039.

β-naphthol.[59] Details of the synthesis of flaviolin trimethyl ether (2 : 5 : 7-trimethoxy-1 : 4-naphthaquinone) *via* 5 : 7-dimethoxytetralone are now



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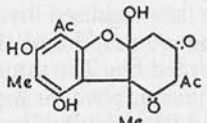

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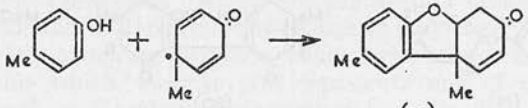


(25)

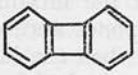
available.[60] The chemistry of naphthazarin has been studied [61] and a new natural colouring matter of this type has been reported.[62]

Non-benzenoid Compounds.—Recognition of the tropylium (*cyclo*heptatrienylium) cation as the aromatic kernel of tropone and tropolone [63] has quickened interest not only in the effect of structural environment upon this kernel but also in the wider issues of what constitutes aromatic character and where it may be found. A timely review of the subject as it concerns non-benzenoid aromatic carbocycles is given by W. Baker and J. F. W. McOmie.[64] Hückel's rule predicts special stability in planar conjugated monocyclic polyolefins which possess $(4n + 2)$ π-electrons (where $n = 0, 1, 2, 3 \ldots$). So far only the aromatic sextet calls for attention: it is attained in the *cyclo*pentadienyl anion, in benzene, and in the tropylium cation. In appropriate cases a formal exocyclic double bond may contribute towards achievement of the annular sextet by supplying a deficient electron, as in the fulvenes (now reviewed by E. D. Bergmann [64]), or by withdrawing a superfluous electron as in tropone. Conceivably also, this process may promote aromaticity in a host of heterocyclic compounds [65] of which a few, like the sydnones, are known but most are unknown and untested. Moreover electron distribution in the dicyclic azulenes shows the tendency (by no means complete) for the transfer of an electron from the seven-membered to the five-membered ring. Such considerations however do not preclude some degree of special stabilisation in other planar cyclic systems,[66] and the problem of a definitive interpretation of aromaticity remains. Similarly, but in a more restricted sense, it is not always easy to distinguish between a true hydroxytropone and an enolisable diketone.

From heats of combustion it is estimated [67] that the change from diphenyl

[59] G. Brunnstrom, *J. Amer. Chem. Soc.*, 1955, **77**, 2463.
[60] J. E. Davies, F. E. King, and J. C. Roberts, *J.*, 1955, 2782.
[61] D. B. Bruce and R. H. Thomson, *J.*, 1955, 1089.
[62] J. H. Lister, C. H. Eugster, and P. Karrer, *Helv. Chim. Acta*, 1955, **88**, 215.
[63] *Ann. Reports*, 1954, **51**, 197.
[64] W. Baker and J. F. W. McOmie in "Progress in Organic Chemistry," ed. J. W. Cook, Butterworths, London, 1955, Vol. 3.
[65] Cf. T. I. Bieber, *Chem. and Ind.*, 1955, 1055.
[66] Cf. J. D. Roberts, A. Streitweiser, and C. M. Regan, *J. Amer. Chem. Soc.*, 1952, **74**, 4579.
[67] R. C. Cass, H. Springall, and (in part) P. G. Quincey, *J.*, 1955, 1188.

to diphenylene (27) is accompanied by a destabilising strain energy of 59 ± 5 kcal./mole, yet diphenylene is a remarkably stable compound. Improved syntheses start from *o*-chloronitrobenzene [68] and from 2 : 2′-dilithiodiphenyl,[69] the latter being converted by mercuric chloride into diphenylenylmercury which yields diphenylene when heated with silver. Diphenylene may be acetylated at position 2 and hence its 2-amino- and 2-carboxy-derivatives have been prepared.[68] The linear dibenzo-derivative, *viz.*, di-2 : 3-naphthylene (1 : 2-3 : 4-di-β-naphtho*cyclo*butadiene), which is synthesised from 2-amino-3-nitronaphthalene,[70] resembles diphenylene in its pale yellow colour and thermal stability, but the angular isomer, di-1 : 2-naphthylene (1 : 2-3 : 4-di-α-naphtho*cyclo*butadiene), which is synthesised from 2 : 2′-di-iodo-1 : 1′-dinaphthyl,[71] is deep-red, and is sensitive to light and to heat, and thus seems to have more *cyclo*butadiene character than the others.



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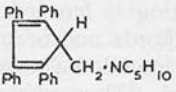
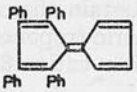
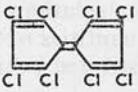


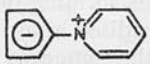
(29)



(30)



(31)



(32)

In harmony with its ability to add Grignard reagents at the exocyclic double bond, 1 : 2 : 3 : 4-tetraphenylfulvene (28; X = CH_2) also adds piperidine but the product (29) can react further affording, as if by a reversed Mannich reaction, tetraphenyl*cyclo*pentadiene and dipiperidinomethane.[72] Although fulvalene remains unknown, a tetraphenyl derivative (30) has been prepared by dehydrating the carbinol obtained from "tetracyclone" (28; X = O) and *cyclo*pentadienylmagnesium bromide : it is a brilliant orange-red solid which forms a red adduct with maleic anhydride.[73] The perchlorofulvalene (31) has also been synthesised by an Ullmann type of reaction applied to hexachloro*cyclo*pentadiene, followed by thermal elimination of chlorine.[74] Unlike the deeply coloured fulvenes which are obtained as condensates of 1 : 2 : 3 : 4-tetrachloro*cyclo*pentadiene with benzaldehydes,[75] the fulvalene is only medium yellow and this is attributed to warping in the molecule (31) due to steric interference between chloro-substituents.

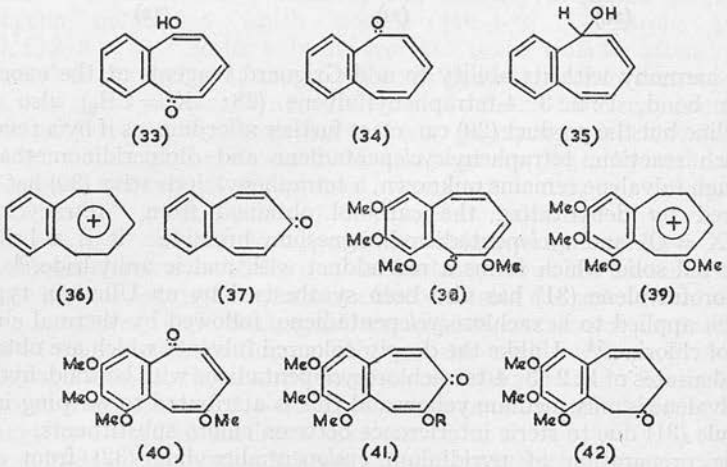
The preparation of pyridinium *cyclo*pentadienylide (32) from *cyclo*pentadiene dibromide and pyridine [76] adds an interesting and accessible

[68] W. Baker, M. P. V. Boarland, and J. F. W. McOmie, *J.*, 1954, 1476.
[69] G. Wittig and W. Herwig, *Chem. Ber.*, 1954, **87**, 1511.
[70] R. F. Curtis and G. Viswanath, *Chem. and Ind.*, 1954, 1174, 1397.
[71] M. P. Cava and J. F. Stucker, *ibid.*, 1955, 446.
[72] D. Taber, E. I. Becker, and P. E. Spoerri, *J. Amer. Chem. Soc.*, 1954, **76**, 776.
[73] E. C. Schreiber and E. I. Becker, *ibid.*, p. 6125.
[74] E. T. McBee, C. W. Roberts, and J. D. Idol, *ibid.*, 1955, **77**, 4942.
[75] E. T. McBee, R. K. Meyers, and C. F. Baranauckas, *ibid.*, p. 86.
[76] D. Lloyd and J. S. Sneezum, *Chem. and Ind.*, 1955, 1221.

member to a class hitherto represented only by the fluorenylides and diazo-*cyclo*pentadiene. The new compound is a red-brown, high-melting solid which affords solutions ranging from colourless in aqueous acid to bluish-purple in light petroleum.

Tropolones, etc.—Carboxynorcaradiene, readily accessible as the ester, provides a new source of tropylium salts.[77] The crude acid is converted *via* the chloride into the azide which, when heated in benzene, yields tropylium *iso*cyanate. Details are now published of the formation of the carboxylated tropylium cation from two isomeric *cyclo*heptatrienecarboxylic acids.[78]

Impressive progress has been made by E. Heilbronner, A. Eschenmoser, and their colleagues in the study of benzotropylium and allied cations.[79, 80] In this series the cation is associated with several pseudo-bases although these are not readily separable from the mixture produced when a solution of the cation is basified. However, oxidation of the mixture or of the solution affords one or more crystalline benzotropones and, contrariwise, individual pseudo-bases are obtained as carbinols when the benzotropones are reduced. Thus, reduction by lithium aluminium hydride applied to the *iso*butyl ether of a known benz*iso*tropolone (33) yields in successive steps the tropone (34) and the oily pseudo-base (35). This last compound dissolves in aqueous acids forming deep-yellow solutions which contain the cation (36) and when oxidised afford equal quantities of the isomeric tropones (34) and (37).[79] In the same way the substituted benzotropylium cation (39), which

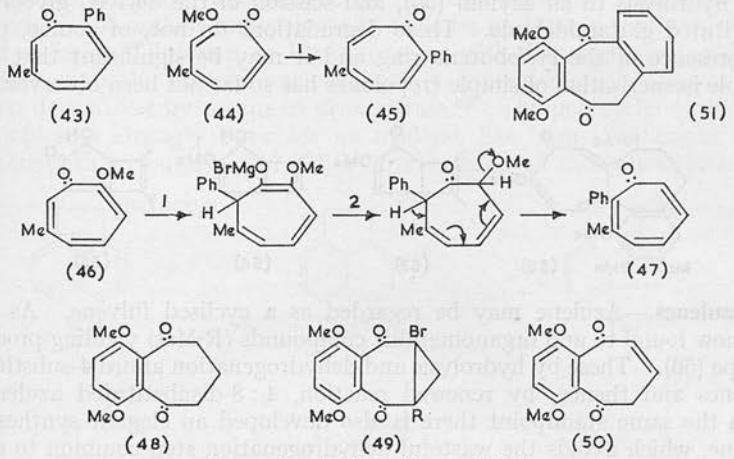


is characterised as the red crystalline picrate and chloroplatinate, is formed from purpurogallin tetramethyl ether (38) *via* the corresponding crystalline carbinol.[80] Oxidation in this case yields the compound (40) which has no readily hydrolysable methoxyl group, but behaves as a substituted tropone

[77] M. J. S. Dewar and R. Pettit, *Chem. and Ind.*, 1955, 199.
[78] A. W. Johnson, A. Langemann, and M. Tišler, *J.*, 1955, 1622.
[79] H. H. Rennhard, E. Heilbronner, and A. Eschenmoser, *Chem. and Ind.*, 1955, 415.
[80] W. H. Schaeppi, R. W. Schmid, E. Heilbronner, and A. Eschenmoser, *Helv. Chim. Acta*, 1955, **38**, 1874.

and regenerates the cation (39) on reduction to the new pseudo-base. These pseudo-bases have a basic strength between those of aniline and *p*-nitroaniline. Attempts to prepare a third pseudo-base reveal in the compound (41) a relatively high resistance to reduction and indeed it is incidental to the interest in this work that a considerable range in properties is found for the three isomers (38), (40), and (41; R = Me). The compound (41) is made available by reduction of the cation (39) with zinc and sulphuric acid : this affords the ketone (42) which is oxidised by selenium dioxide to the tropolone (41).

Electrophilic substitution of 3-hydroxytropone occurs at position 2 wherein a nitro-substituent may be reduced, an amino-group diazotised, but a halogen atom appears to be rather unreactive.[81] The synthesis of 4-hydroxytropone, effected *via* 4-bromotropone by bromination of suberone,[82] or *via* 4-methoxytropone from 3 : 6-dimethoxy*cyclo*heptatrienecarboxylic acid,[83] now completes the set of three hydroxytropones of which tropolone was the first. The new isomer more nearly resembles tropolone than does tropone or 3-hydroxytropone. Results of considerable significance have attended the study of organometallic compounds in reaction with tropolones.[84] Whereas phenyl-lithium reacts with the copper complex of β-methyltropolone to form a normal type of product, *viz.*, 6-methyl-2-phenyltropone (43), the two methyl ethers (44) and (46) react with phenylmagnesium



Reagents : 1, PhMgBr. 2, H^+.

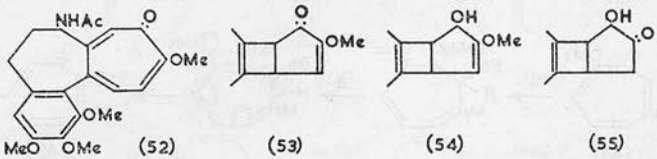
bromide yielding 5-methyl- and 3-methyl-2-phenyltropone (45) and (47), respectively. For these reactions a mechanism involving 1 : 8-addition is proposed and a similar process may explain the abnormal course of certain amination reactions reported last year. The isomeric methylphenyltropones are structurally identified by conversion into methyldiphenyl-2-carboxylic acids and hence into the known methylfluorenones. Such rearrangement into a benzenoid carboxylic acid remains the most direct

[81] A. W. Johnson and M. Tišler, *J.*, 1955, 1841.
[82] T. Nozoe, T. Mukai, Y. Ikegami, and T. Toda, *Chem. and Ind.*, 1955, 66.
[83] R. S. Coffey, R. B. Johns, and A. W. Johnson, *ibid.*, p. 658.
[84] R. D. Haworth and P. B. Tinker, *J.*, 1955, 911.

method of orienting tropone derivatives and by the use of labelled compounds it is shown [85] that the carbon atom of the carboxyl group is derived from that of the carbonyl group in the tropone.

Bromination–dehydrobromination of compound (48) [86] and of its dienol acetate [87] yields products which are formulated as (49; R = H or Br) and (50), respectively. On this basis the diketone (50) appears to be surprisingly unlike the enolised parent compound (33) and to have fewer enol (tropolone) properties than its benzo-homologue (51) which is reported [88] to form an enol methyl ether.

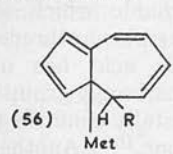
The configuration of colchicine is elucidated through successive ozonolysis and oxidation to *N*-acetyl-L-glutamic acid.[89] Colchicide, the tropone corresponding to colchicine (52), is obtained from the latter by reaction with methanethiol and desulphurisation of the resultant sulphide.[90] When exposed to sunlight in aqueous solution and in absence of air, colchicine is isomerised to a mixture of α-, β-, and γ-lumicolchicines [91] of which the last two are closely allied and occur with colchicine in the plant.[92] The tropolone properties of colchicine are lacking in β- and γ-lumicolchicine which are considered to be stereoisomers formed by bond rearrangement (52) ⟶ (53) in the tropolone ring.[93] The compounds behave as diolefinic tetracyclic ketones wherein the methoxylated *cyclo*pentenone structure is in each case disclosed by successive reduction with sodium borohydride to an enol ether (54), hydrolysis to an acyloin (55), and scission of the derived glycol to a substituted glutaraldehyde. These degradations do not, of course, prove the presence of the *cyclo*butene ring and it may be significant that comparable isomerisation of simple tropolones has so far not been observed.

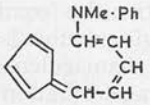


Azulenes.—Azulene may be regarded as a cyclised fulvene. As such it is now found to add organometallic compounds (R·Met) yielding products of type (56). These by hydrolysis and dehydrogenation afford 4-substituted azulenes and thence, by renewed reaction, 4 : 8-disubstituted azulenes.[94] From the same standpoint there is also developed an elegant synthesis of azulene, which avoids the wasteful dehydrogenation step common to other methods. Thus the fulvene (57), obtained by condensing *cyclo*pentadiene with the glutacondialdehyde derivative (58) (prepared from 2 : 4-dinitro-

[85] W. von E. Doering and D. B. Denney, *J. Amer. Chem. Soc.*, 1955, **77**, 4619.
[86] A. J. S. Sorrie and R. H. Thomson, *J.*, 1955, 2238.
[87] *Idem, ibid.*, p. 2233.
[88] *Idem, ibid.*, p. 2244.
[89] H. Corrodi and E. Hardegger. *Helv. Chim. Acta*, 1955, **38**, 2030; cf. G. Muller and L. Velluz, *Bull. Soc. chim. France*, 1955, 1452.
[90] H. Rapoport and J. B. Lavigne, *J. Amer. Chem. Soc.*, 1955, **77**, 667; cf. L. Velluz and G. Muller, *Bull. Soc. chim. France*, 1954, 755; 1955, 198.
[91] R. Grewe and W. Wulf, *Chem. Ber.*, 1951, **84**, 621.
[92] F. Šantavý, *Coll. Czech. Chem. Comm.*, 1951, **16**, 655.
[93] E. J. Forbes, *J.*, 1955, 3864.
[94] K. Hafner and H. Weldes, *Angew. Chem.*, 1955, **67**, 302, 348.

phenylpyridinium chloride and methylaniline), gives a 60% yield of azulene when heated *in vacuo*.[95]





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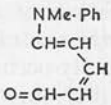
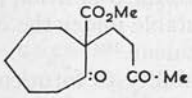


(58)



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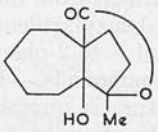

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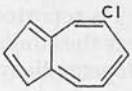
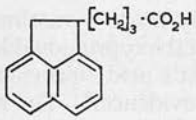


(61)

Other new types of azulene synthesis include pinacone-reduction of the diketonic ester (59) which yields the lactone (60), transformable into 1-methylazulene.[96] Moreover in parallel with the well-known ring-expansion of pyrrole and indole to 3-chloro-pyridine and -quinoline, respectively, indenylsodium reacts with chloroform yielding 2-chloronaphthalene and this is accompanied by a product regarded as the chloroazulene (61).[97] W. Treibs has outlined recent advances made by his research school: these include the synthesis of azulene and polycyclic azulenes by high-temperature interaction of indane, fluorene, or benzofluorenes with diazomethane; radical-substitution in the azulene nucleus; and a general method for nitrating azulene derivatives by means of urea nitrate.[98] The polycyclic compound (63), which is strongly basic for an azulene, has been synthesised from γ-1-acenaphthenylbutyric acid (62) by cyclisation and subsequent standard



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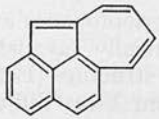

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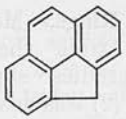

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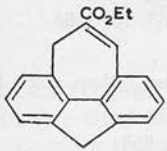

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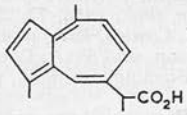
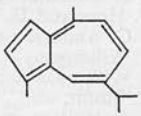


(66)



(67)

procedure.[99] Surprisingly it is also the parent hydrocarbon of two azulene-carboxylic esters obtained by the action of diazoacetic ester on the *cyclo*-pentaphenanthrene (64): these esters are accompanied by another which

[95] K. Ziegler and K. Hafner, *Angew. Chem.*, 1955, **67**, 301.
[96] D. Lloyd and F. Rowe, *J.*, 1954, 4232.
[97] W. E. Parham and H. E. Reiff, *J. Amer. Chem. Soc.*, 1955, **77**, 1177.
[98] W. Treibs, *Angew. Chem.*, 1955, **67**, 76.
[99] D. H. Reid, W. H. Stafford, and J. P. Ward, *J.*, 1955, 1193.

survives the conditions of the reaction as a dihydroazulene, *viz.* (65). The multiple attack by diazoacetic ester in these reactions is in contrast with the simple course of oxidation by osmium tetroxide which selectively hydroxylates the central bridging double-bond of the phenanthrene (64).

The naturally-occurring chamazulenecarboxylic acid has now been identified as (66) through transformation of its carboxyl group into the methyl group of guaiazulene (67) [100] and this incidentally confirms the structure of chamazulene as 7-ethyl-1 : 4-dimethylazulene.[101] Another case of migration of an *iso*propyl substituent from the 1- to the 2-position in azulene derivatives is reported,[102] but 1-benzylazulene is stable under the conditions which effect the similar change of 1- to 2-phenylazulene.[103]

Di*cyclo*pentadienyl–metal Compounds.—The ability to form compounds analogous to ferrocene in structure is common to the transitional elements which have two vacant or singly occupied *d*-orbitals available for bonding. Such compounds incorporating all the transition metals have been described,[104] and their electronic structure discussed.[105] New types are portended by the discovery of mono- and di-*cyclo*pentadienyl–metal carbonyls.[106] Phenyl-substituted *cyclo*pentadienes afford phenyl-substituted ferrocenes [107] and from indene, di-indenyl derivatives of iron and cobalt have been prepared.[108] Ferrocene may be metalated by means of *n*-butyllithium and thence its mono- and di-carboxylic acids are obtained.[109] It undergoes arylation to mono- and di-arylferrocenes by reaction with aryldiazonium salts or *N*-nitrosoacetanilide; [110, 111] sulphonation by sulphuric acid; and in hydrogen fluoride condensation with aldehydes or acetylation by acetic anhydride.[112] A comprehensive review of the subject has been published.[113]

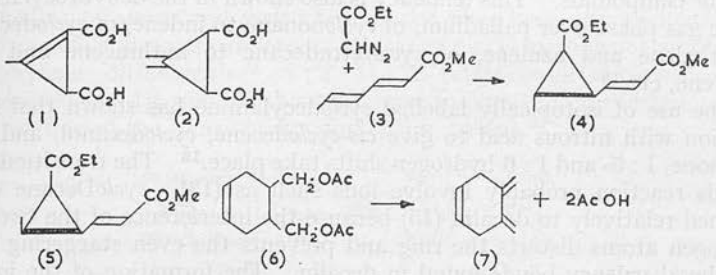
J. D. L.

6. ALICYCLIC COMPOUNDS.

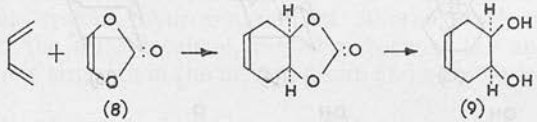
Small Rings.—Monoalkyl*cyclo*propanes are easily made by a three-stage synthesis from the commercially available 3-ethoxypropionaldehyde.[1] Infrared studies [2] support the structure (1) for Feist's acid; previously the structure (2) was advanced from *X*-ray diffraction evidence.[3] The reaction of ethyl diazoacetate with methyl sorbate (3) affords the *cyclo*propanes (4) and (5), a concerted addition to the $\gamma\delta$-double bond thus being excluded.[4]

[100] E. Stahl, *Chem. Ber.*, 1954, **87**, 1626.
[101] A. Meisels and A. Weizmann, *J. Amer. Chem. Soc.*, 1953, **75**, 3865.
[102] W. Herz and B. E. Cleare, *ibid.*, 1955, **77**, 2318.
[103] A. G. Anderson and E. J. Cowles, *ibid.*, p. 4617.
[104] G. Wilkinson, P. L. Pauson, and F. A. Cotton, *J. Amer. Chem. Soc.*, 1954, **76**, 1970; G. Wilkinson and J. M. Birmingham, *ibid.*, p. 4281.
[105] W. Moffit, *ibid.*, p. 3386.
[106] B. F. Hallam and P. L. Pauson, *Chem. and Ind.*, 1955, 653.
[107] P. L. Pauson, *J. Amer. Chem. Soc.*, 1954, **76**, 2187.
[108] P. L. Pauson and G. Wilkinson, *ibid.*, p. 2024.
[109] R. A. Benkeser, D. Goggin, and G. Schroll, *ibid.*, p. 4025.
[110] G. D. Broadhead and P. L. Pauson, *J.*, 1955, 367.
[111] V. Weinmayr, *J. Amer. Chem. Soc.*, 1955, **77**, 3012.
[112] *Idem, ibid.*, p. 3009.
[113] P. L. Pauson, *Quart. Rev.*, 1955, **9**, 391.
[1] J. T. Gragson, K. W. Greenlee, J. M. Derfer, and C. E. Boord, *J. Org. Chem.*, 1955, **20**, 275.
[2] G. R. Boreham, F. R. Goss, and G. J. Minkoff, *Chem. and Ind.*, 1955, 1354.
[3] D. Lloyd, T. C. Downie, and J. C. Speakman, *ibid.*, 1954, 492.
[4] S. H. Harper and H. W. B. Reed, *J.*, 1955, 779.

1 : 2-Dimethylene*cyclo*butane has been obtained by pyrolysis of the dimethohydroxide of 1 : 2-bis(dimethylaminomethyl)*cyclo*butane.[5] Many thermodynamically unstable exocyclic dienes [*e.g.*, 4 : 5-dimethylene*cyclo*hexene (7), which is isomeric with *o*-xylene] have been made by pyrolysis



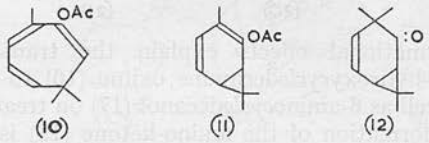
of the appropriate diacetates, *e.g.*, (6);[6,7] they are likely to prove particularly useful in the synthesis of polycyclic aromatic compounds. Their ultraviolet absorption does not obey Woodward's rules.[8]



Studies are reported on the effect of ring strain on the ultraviolet absorption of αβ-unsaturated ketones,[9] and of ring size on the keto–enol equilibria of α-diketones.[10]

Vinylene carbonate (1 : 3-dioxol-2-one) (8) promises to make easily available many *cis*-glycols, such as *cyclo*hex-4-ene-1 : 2-diol (9), which can be dehydrated to aromatic compounds.[11]

Medium and Large Rings.—*cyclo*Heptatriene and *bicyclo*heptadiene structures may be distinguished by measurements of nuclear magnetic resonance. Thus the enol acetate of eucarvone is shown to have the formula



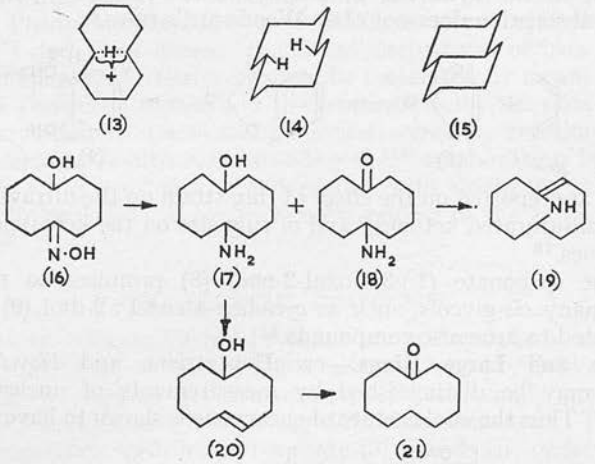
(10) and not (11), in spite of the fact that when ozonised it affords *cis*-caronic acid in more than 50% yield. On the other hand, methylation of sodioeucarvone gives the methylated carene derivative (12).[12]

The base-catalysed decomposition of tropinone methiodide yields *cyclo*-

[5] A. T. Blomquist and J. A. Verdol, *J. Amer. Chem. Soc.*, 1955, **77**, 1806.
[6] W. J. Bailey and J. Rosenberg, *ibid.*, p. 73; W. J. Bailey, J. J. Hewitt, and C. King, *ibid.*, p. 357; W. J. Bailey, C.-W. Liao, and G. H Coleman, *ibid.*, p. 990; W. J. Bailey, J. Rosenberg, and L. J. Young, *ibid.*, p. 1163.
[7] W. J. Bailey and W. B. Lawson, *ibid.*, p. 1606.
[8] R. B. Woodward, *ibid.*, 1941, **63**, 1123.
[9] W. M. Schubert and W. A. Sweeney, *ibid.*, 1955, **77**, 2297.
[10] G. Hesse and G. Krehbiel, *Annalen*, 1955, **593**, 35.
[11] M. S. Newman and R. W. Addor, *J. Amer. Chem. Soc.*, 1955, **77**, 3789.
[12] E. J. Corey, H. J. Burke, and W. A. Remers, *ibid.*, p. 4941.

hepta-2 : 4- and 3 : 5-dienones, the ultraviolet absorption of the latter being anomalous.[13] Similar decomposition of homo*pseudo*pelletierine methiodide affords 4 : 5 : 3*a* : 7*a*- and 4 : 5 : 6 : 3*a*-tetrahydroindan-5-one,[14] showing the tendency of nine-membered and larger rings to change into less strained bicyclic compounds. This tendency is also shown in the dehydrocyclisation, in the gas phase over palladium, of *cyclo*nonane to indene, of *cyclo*decane to naphthalene and azulene, of *cyclo*tetradecane to anthracene and phenanthrene, etc.[15]

The use of isotopically labelled *cyclo*decylamine has shown that in the reaction with nitrous acid to give *cis-cyclo*decene, *cyclo*decanol, and *cyclo*decanone, 1 : 5- and 1 : 6-hydrogen shifts take place.[16] The transition states of this reaction probably involve ions such as (13). *cyclo*Decane (14) is strained relatively to decalin (15) because the interference of the two inner hydrogen atoms distorts the ring and prevents the even staggering of the peripheral valency bonds found in decalin. The formation of the ion (13) from a *cyclo*decyl derivative relieves some of this strain.

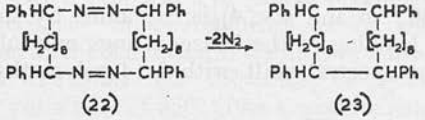


Similar conformational effects explain the transannular hydrogen transfer causing 6-hydroxy*cyclo*decanone oxime (16) to give 6-amino*cyclo*decanone (18) as well as 6-amino*cyclo*decanol (17) on treatment with sodium in butanol. The formation of the amino-ketone (18) is inferred from the isolation of the bicyclic amine (19), an example of the violation of Bredt's rule possible with large rings.[17] Hofmann degradation of the amino-alcohol (17) affords *cyclo*dec-5-enol (20); on treatment with palladised charcoal this gives *cyclo*decanone (21) by transannular hydrogen transfer. A mixture of Δ^{1}- and $\Delta^{1(9)}$-octalins is obtained when the toluene-*p*-sulphonate of the unsaturated alcohol (20) is heated in diethylaniline at 160°.[18]

[13] J. Meinwald, S. L. Emerman, N. C. Yang, and G. Büchi, *J. Amer. Chem. Soc.*, 1955, **77**, 4401.
[14] J. Meinwald and M. Koskenkyla, *Chem. and Ind.*, 1955, 476.
[15] V. Prelog, V. Boarland, and S. Polyák, *Helv. Chim. Acta*, 1955, **38**, 434.
[16] V. Prelog, H. J. Urech, A. A. Bothner-By, and J. Würsch, *ibid.*, p. 1095.
[17] A. C. Cope, R. J. Cotter, and G. G. Roller, *J. Amer. Chem. Soc.*, 1955, **77**, 3590.
[18] *Idem, ibid.*, p. 3594.

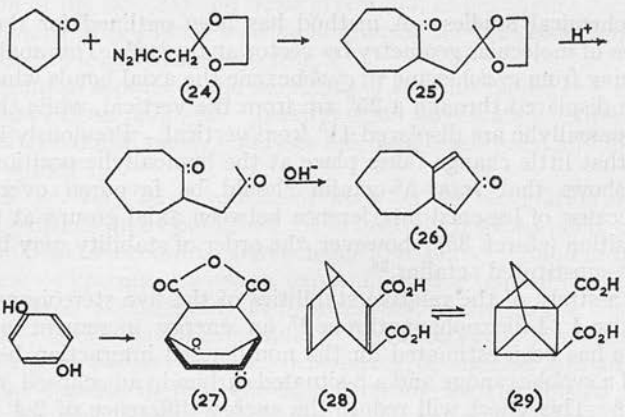
*trans-cyclo*Nonene and *-cyclo*decene are isomerised to the more stable *cis*-compounds in presence of naphthalene-2-sulphonic acid as a catalyst.[19] *cis-cis-* and *cis-trans-cyclo*Deca-1 : 3-diene have been prepared. The former compound shows no absorption maximum in the region 215—230 mμ, indicating that its double bonds are not coplanar.[20]

*cyclo*Hexadecanone and *cyclo*octadecanone have been prepared from the readily available diametric diketones by Raney nickel hydrogenolysis of their monoethylene dithioketals.[21] 1 : 2 : 11 : 12-Tetraphenyl*cyclo*eicosane (23) was obtained in 72% yield without recourse to high dilution techniques by decomposition of the bisazo-compound (22) in xylene.[22]



(22) (23)

Polycyclic Compounds.—*trans*-9-Carboxy-,[23] -9-amino-,[23] and -9-methyldecalin, and *cis*-9-methyldecalin [24] have been made by stereospecific routes. While hydrogenation of steroidal 4-en-3-ones gives products having the *cis*-fused A/B ring system, hydrogenation of 10-ethoxycarbonyl-2-oxo-$\Delta^{1(9)}$-octalin affords the *trans*-decalone, probably because the angular ethoxycarbonyl group is larger than the methyl group and blocks adsorption on the



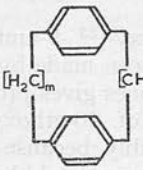
(24) (25) (26) (27) (28) (29)

catalyst.[23, 25] Treatment of *cyclo*hexanone with the diazo-compound (24) gives the ring-expanded ketone (25), whence *bicyclo*[5 : 3 : 0]dec-1(10)-en-9-one (26) may be obtained by treatment with acid and then alkali.[26]

[19] A. C. Cope, D. C. McLean, and N. A. Nelson, *J. Amer. Chem. Soc.*, 1955, **77**, 1628; A. T. Blomquist and A. Goldstein, *ibid.*, p. 1001.
[20] *Idem*, *ibid.*, p. 998.
[21] A. T. Blomquist, J. Prager, and J. Wolinsky, *ibid.*, p. 1804.
[22] C. G. Overberger and M. Lapkin, *ibid.*, p. 4651.
[23] W. G. Dauben, R. C. Tweit, and R. L. MacLean, *ibid.*, p. 48.
[24] W. G. Dauben, J. B. Rogan, and E. J. Blanz, *ibid.*, 1954, **76**, 6384; A. S. Dreiding and A. J. Tomasewski, *ibid.*, 1955, **77**, 168.
[25] *Idem*, *ibid.*, p. 411.
[26] A. M. Islam and R. A. Raphael, *J.*, 1955, 3151.

The reaction of quinol with maleic anhydride at 190° affords the *bicyclo*-[2 : 2 : 2]octane derivative (27).[27] Convenient routes to *bicyclo*[3 : 2 : 1]oct-2-ene and *bicyclo*[3 : 2 : 2]non-2-ene have been described,[28] and *bicyclo*-[2 : 2 : 2]octa-2 : 5-diene has been made.[29] Ultraviolet irradiation of the *bicyclo*[2 : 2 : 1]heptadiene (28) affords an isomer formulated as (29); the reaction is reversed by heating the product with palladium.[30]

Although [6 : 6]-*para*cyclophane (30; $m = n = 6$) undergoes Friedel-Crafts acetylation in both rings,[31] [4 : 4]*para*cyclophane (30; $m = n = 4$) affords only a monoacetyl compound.[32] This transannular deactivation is attributed to overlap of the π-orbitals of the two benzene rings. The shift in the ultraviolet absorption peak to longer wavelengths with the smaller *para*cyclophanes (30; m and $n < 4$) is explained by similar transannular effects. However, bending of the benzene rings may also be involved, as shown by a similar spectral shift with 1 : 4-polymethylenebenzenes (31) when $n < 10$.[33]



(30)

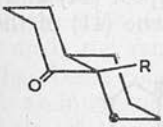
(31)

Stereochemical Studies.—A method has been outlined for the accurate calculation of molecular geometry by vector analysis.[34] This analysis shows that in going from *cyclo*hexane to *cyclo*hexene the axial bonds which become allylic are displaced through a 25° arc from the vertical, while those which become homoallylic are displaced 11° from vertical. Previously it has been assumed that little change takes place at the homoallylic positions.[35] The analysis shows that *trans*-Δ^2-octalin should be favoured over *trans*-Δ^1-octalin because of lessened interference between axial groups at the 8- and the 10-position (cf. ref. 35); however, the order of stability may be reversed in heavily substituted octalins.[36]

From a study of the relative stabilities of the five stereoisomeric forms of perhydro-1 : 4-dioxophenanthrene,[37] an energy increment of 0·8—1·2 kcal./mole has been estimated for the non-bonded interaction between the oxygen of a *cyclo*hexanone and a β-situated carbon in an eclipsed (equatorial) position.[38] This effect will reduce the energy difference of 2·4 kcal./mole

[27] R. C. Cookson and N. S. Wariyar, *Chem. and Ind.*, 1955, 915.
[28] K. Alder, H. Krieger, and H. Weiss, *Chem. Ber.*, 1955, **88**, 144.
[29] J. Hine, J. A. Brown, L. H. Zalkow, W. E. Gardner, and M. Hine, *J. Amer. Chem. Soc.*, 1955, **77**, 594.
[30] S. J. Cristol and R. L. Snell, *ibid.*, 1954, **76**, 5000.
[31] D. J. Cram and J. Abell, *ibid.*, 1955, **77**, 1179.
[32] D. J. Cram and R. W. Kierstead, *ibid.*, p. 1186.
[33] D. J. Cram, N. L. Allinger, and H. Steinberg, *ibid.*, 1954, **76**, 6132.
[34] E. J. Corey and R. A. Sneen, *ibid.*, 1955, **77**, 2505.
[35] *Ann. Reports*, 1954, **51**, 172.
[36] G. Stork and A. W. Burgstahler, *J. Amer. Chem. Soc.*, 1955, **77**, 5068; cf. A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, 1955, **38**, 1890 (too late for review here).
[37] P. A. Robins and J. Walker, *J.*, 1954, 3960.
[38] *Idem, ibid.*, 1955, 1789; *Chem. and Ind.*, 1955, 772.

between *trans*- and *cis*-decalin to 1·2—1·6 kcal./mole between *trans*-α-decalone (32; R = H) (in which the oxygen eclipses the β-carbon atom shown as a dot) and *cis*-α-decalone (33; R = H). The effect also operates



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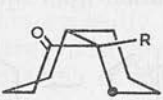

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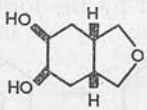

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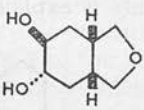


(35)

to favour a slightly higher proportion of axially oriented bulky groups such as benzyl [39] and bromine [40] in α-substituted *cyclo*hexanones. An angular methyl group diminishes the difference in stability of *trans*- and *cis*-decalin; [41] and equilibration of the *trans*- (32; R = Me) or *cis*-9-methyldecal-1-one (33; R = Me) over palladium at 250° gives a mixture containing 60% of the *cis*- and 40% of the *trans*-compound.[42]

The functional groups of the *cis*-(±)-3-amino*cyclo*hexanecarboxylic acid appear to be diaxial in the solid state but diequatorial in aqueous solution.[43] Evidently the very large coulombic attractions favouring the diaxial conformation of the zwitterion are more than compensated by the increased ionic solvation possible in the diequatorial conformation.

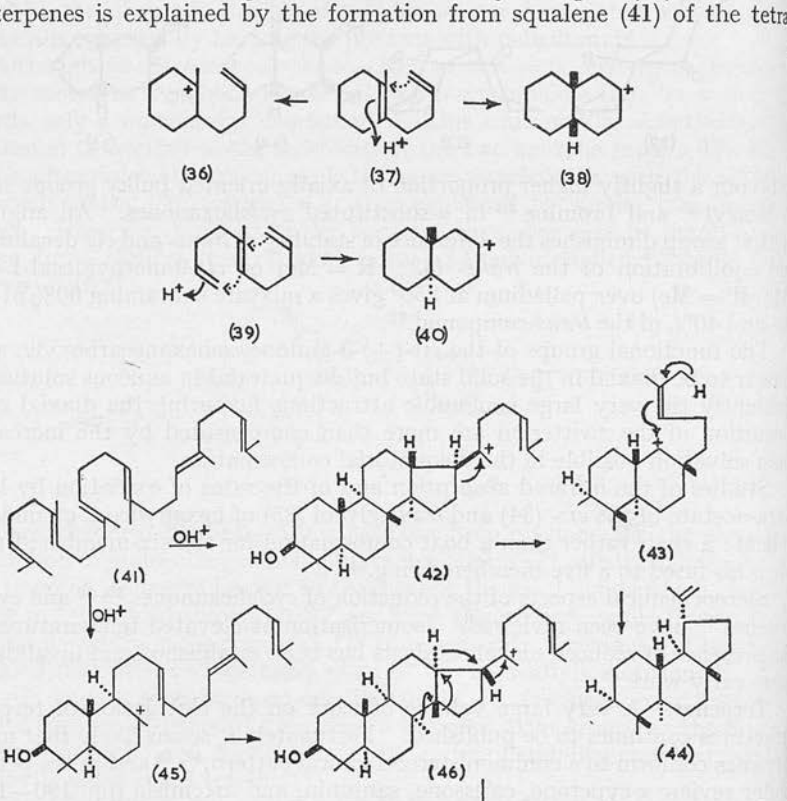
Studies of the infrared absorption and of the rates of oxidation by lead tetra-acetate of the *cis*- (34) and *trans*-glycol (35) of hexahydro-2-oxaindane indicate a chair rather than a boat conformation for the six-membered ring when *cis*-fused to a five-membered ring.[44]

Stereochemical aspects of the reduction of *cyclo*hexanones [45, 46] and *cyclo*hexenes [46] have been reviewed. Isomerisation at elevated temperatures in the presence of reduced nickel catalysts has been established and invalidates some early work.[46]

Terpenes.—A very large volume of work on the elucidation of terpene structures continues to be published. Fortunately it seems likely that most terpenes conform to a common stereochemical pattern,[47, 48] and in the period under review α-cyperone, carissone, santonin, and artemisin (pp. 190—193) have been related to eudesmol, itself previously related to the steroids and triterpenes.[48] Stork and Burgstahler [36] have recently presented a biogenetic theory to explain the stereochemistry of steroids and triterpenes, which is also capable in principle of accounting for the smaller terpenes. They point out that while ring closure of the monocyclic 1 : 5-diene (37), by a concerted *trans*-attack on the cyclic double bond of a proton and of the nucleophilic carbon of the second double bond, should lead *via* the ion (38)

[39] E. J. Corey, T. H. Topie, and W. A. Wozniak, *J. Amer. Chem. Soc.*, 1955, **77**, 5415.
[40] E. J. Corey and H. J. Burke, *ibid.*, p. 5418.
[41] *Ann. Reports*, 1952, **49**, 177.
[42] A. Ross, P. A. S. Smith, and A. S. Dreiding, *J. Org. Chem.*, 1955, **20**, 905.
[43] F. R. Hewgill and P. R. Jefferies, *J.*, 1955, 2767.
[44] E. L. Eliel and C. Pillar, *J. Amer. Chem. Soc.*, 1955, **77**, 3600; cf. S. J. Angyal and C. G. Macdonald, *J.*, 1952, 686.
[45] R. Cornubert, G. Barraud, M. Cormier, M. Descharmes, and H. G. Eggert, *Bull. Soc. chim. France*, 1955, 400.
[46] E. G. Peppiatt and R. J. Wicker, *Chem. and Ind.*, 1955, 716, 747; *J.*, 1955, 3122.
[47] L. Ruzicka, *Experientia*, 1953, **9**, 357; *Ann. Reports*, 1953, **50**, 209.
[48] Cf. *Ann. Reports*, 1954, **51**, 207.

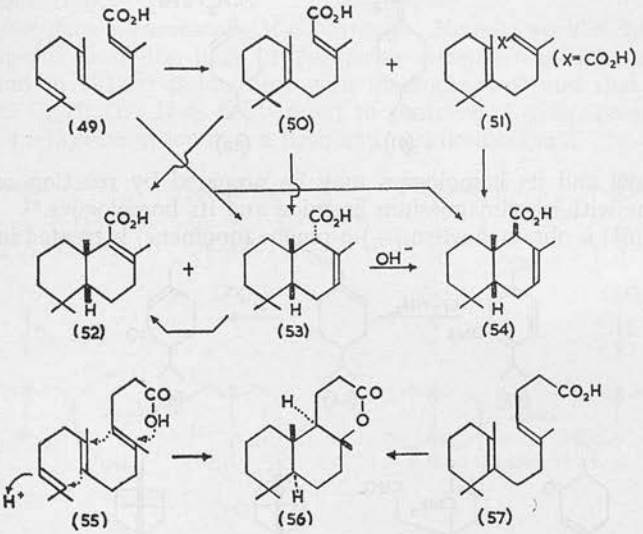
to a *cis*-decalin derivative, cyclisation of the acyclic triene (39) by a concerted mechanism should lead *via* the ion (40) to a *trans*-decalin. [A non-concerted cyclisation *via* the carbonium ion (36) would be expected to give a mixture of *cis*- and *trans*-decalin.] The stereochemistry of lupeol (44) and related triterpenes is explained by the formation from squalene (41) of the tetra-



cyclic intermediate (42) by a concerted reaction, followed by ring expansion giving the ion (43), and further cyclisation. On the other hand, the formation of lanosterol (48) and of the steroids is considered to involve a stereochemically distinctive tetracyclic intermediate (46), formed by a sequence of two concerted reactions with (45) as the intermediate carbonium ion. The tetracyclic intermediate (46) then undergoes a stereospecific series of concerted 1 : 2-shifts to give the isomeric carbonium ion (47), which eliminates a proton to form lanosterol (48).

Unfortunately, attempts to effect these stereospecific cyclisations in the

laboratory [36] have met with only partial success. Farnesic acid (49) is converted by boron trifluoride–ether in benzene at temperatures below 5° into the monocyclic compounds (50) and (51), which are further transformed by the same reagent at 40° into the *cis*-octalins (52), (53), and (54), as predicted. However, the acids (53) and (54) were also formed directly by cyclisation of farnesic acid, the reaction evidently involving monocyclic intermediates rather than being concerted. The epimerisation (at 150°) of the acid (53) to (54) is an interesting example of an equatorial substituent's going into a more stable axial position.



Cyclisation of farnesylacetic acid (55) by stannic chloride or bromide gave the expected *trans*-compound, (±)-ambreinolide (56), in very low yield. However the monocyclic compound (57) also gave this product, presumably by a non-concerted reaction.

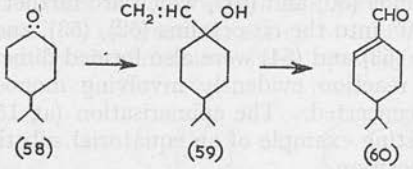
The formation of the terpenes in Nature in one enantiomeric form must involve the mediation of asymmetric reagents. If these are enzymes, the synthesis most probably takes place in aqueous solution, in which crumpled forms, such as (41), (49), (50), (51), and (55), necessary to cyclisation will be favoured because of the smaller areas of hydrocarbon–water interface for the molecules. In benzene and other organic solvents the more extended conformations should preponderate.

A useful account of the use of *X*-ray methods in the elucidation of the structures of terpenes has been published.[49]

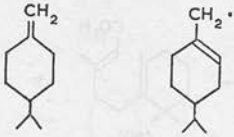
Monoterpenes.—A synthesis[50] of (±)-phellandral (60) starts from dihydrocryptone (58) which is condensed with sodium acetylide. The acetate of the alcohol (*trans* C:C and Pr^i) formed is reduced to the ethylenic alcohol (59) and ozonised. Dehydration is effected during the formation of the 2 : 4-

[49] J. M. Robertson, *Roy. Inst. Chem. Lectures*, 1954, No. 6.
[50] D. T. C. Gillespie, P. R. Jefferies, A. K. Macbeth, and M. J. Thompson, *J.*, 1955, 665.

dinitrophenylhydrazone of the product, the derivative of (±)-phellandral being obtained.

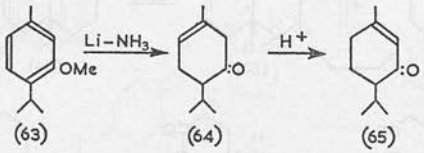


(58) (59) (60)

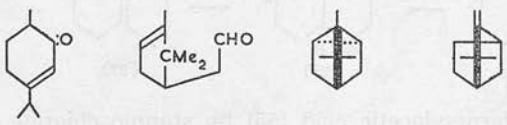


(61) (62)

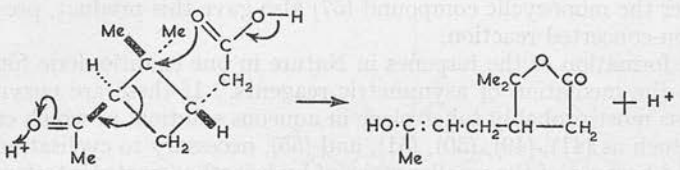
Linaloöl and its homologues may be prepared by reaction of methyl heptenone with vinylmagnesium bromide and its homologues.[51] *p*-Menth 1(7)-ene (61) is obtained when (—)-β-pinene (nopinene) is treated in presenc



(63) (64) (65)



(66) (67) (70) (71)



(68) (69)

of ultraviolet light and peroxides with trichlorosilane, the carvomenthe derivative (62; R = Cl) so produced being treated with methylmagnesiu iodide to give the silane (62; R = Me) and the latter is then decompos with acetic acid.[52]

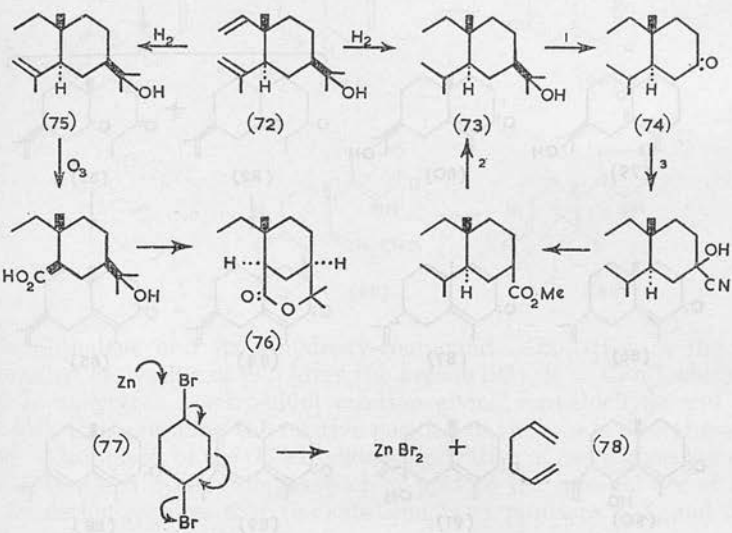
Reduction of thymyl methyl ether (63) with lithium in liquid ammon affords the unsaturated ketone (64), isomerised by acid to piperitone (6 Carvacryl methyl ether similarly affords carvenone (66).[53]

[51] H. Normant, *Compt. rend.*, 1955, **240**, 631.
[52] E. Frainnet and R. Calas, *ibid.*, p. 203.
[53] G. Dupont, R. Dulou, and P. Crabbe, *Bull. Soc. chim. France*, 1955, 621.

Treatment of α-pinene with monoperphthalic acid gives 48% yield of epoxide, and a fair yield of campholenaldehyde (67) by isomerisation of the epoxide. Limonene monoepoxide is transformed by acid to carvenone (66).[54] The mechanism of the well-known [55] acid-catalysed rearrangement of pinonic acid (68) to homoterpenyl methyl ketone (shown as an enol; 69) has been discussed.[56] Complete conversion of both the *trans*- and the *cis*-acid can be effected by trichloroacetic acid.

Tricyclene (70) and camphene (71), simultaneously obtained by isomerism of α-pinene, have similar heats of combustion and stabilities and can be interconverted.[57]

Sesquiterpenes.—Juniperol (*Macrocarpol*). Recent work [58] has shown that juniperol, from the bark of *Juniperus communis* L., investigated in 1909 [59] and in 1913,[60] is identical with macrocarpol,[61] and that the true formula is $C_{15}H_{26}O$. It is dehydrated to junipene, $C_{15}H_{24}$, possessing an exocyclic methylene group and a trisubstituted double bond.



Reagents: 1, CrO_3. 2, MeMgCl. 3, KCN.

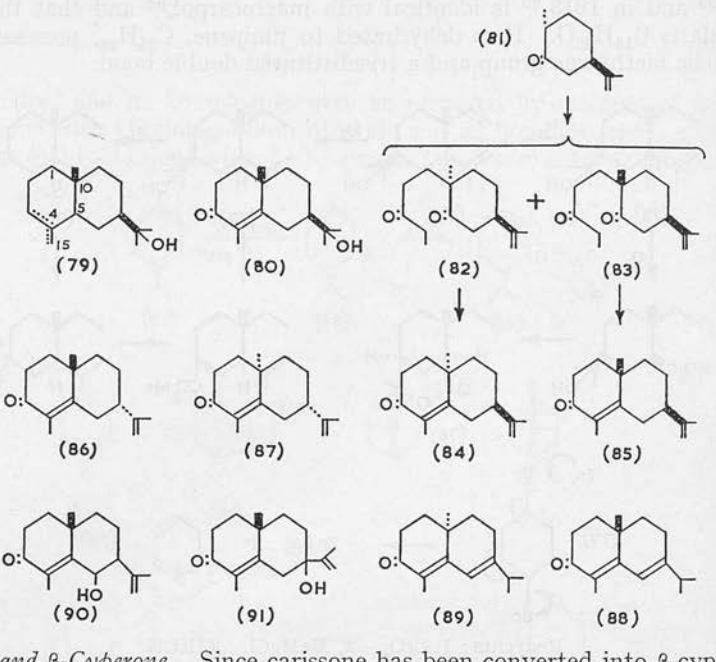
Elemol [62] has most probably the structure (72).[63] The hydroxypropyl nature of the side chain has been proved by removing it from the tetrahydro-compound (73) to give the *cyclo*hexanone (74), and then restoring it by the

[54] E. E. Royals and L. L. Harrell, *J. Amer. Chem. Soc.*, 1955, **77**, 3405.
[55] (Sir) John Simonsen and L. N. Owen, "The Terpenes," Cambridge Univ. Press, 1949, Vol. II, p. 115.
[56] C. L. Arcus and G. J. Bennett, *J.*, 1955, 2627.
[57] G. Swann, *Chem. and Ind.*, 1954, 1516.
[58] H. Erdtman and B. R. Thomas, *ibid.*, 1955, 384.
[59] H. Ramsay, *Z. Kryst. Min.*, 1909, **46**, 281.
[60] G. Mattsson, *Bidr. Finl. Nat. Folk*, 1913, **H.72**, 1. Beilstein's "Handbuch," Vol. 6, 2nd. Suppl., p. 516.
[61] L. H. Briggs and M. D. Sutherland, *J. Org. Chem.*, 1942, **7**, 397; C. W. Brandt and B. R. Thomas, *New Zealand J. Sci. Technol.*, 1951, **33**, *B*, 30.
[62] *Ann. Reports*, 1954, **51**, 207.
[63] V. Sýkora, V. Herout, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1955, **20**, 220.

synthetic route shown.[63] The stereochemistry indicated is probable from analogy with the bicyclic sesquiterpenes; some support is given by formation of the lactone (76) from dihydroelemol (75).[64] An analogy for the ring scission postulated in the biogenesis of elemol [47] is given by the formation of hexa-1 : 5-diene (78) from 1 : 4-dibromo*cyclo*hexane (77).[65]

Sagittol [66] from the steam-volatile oil of *Balsamorrhiza sagittata* (Pursh) Nutt has been identified [67] as eudesmol (79).

Carissone.[68] Reaction of eudesmol with nitrosyl chloride, and treatment of the product with sodium ethoxide, afford the oxime of carissone, which is consequently shown to have the stereochemistry depicted in (80).[69] The reaction further shows that eudesmol, even when chromatographically pure, is a mixture of the Δ^3- and $\Delta^{4(15)}$-compounds.



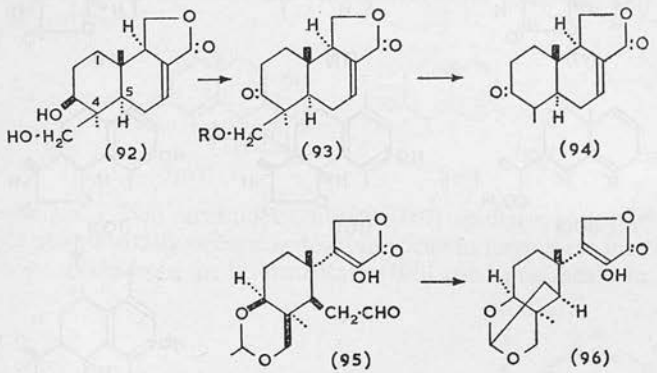
α- and β-Cyperone. Since carissone has been converted into β-cyperone (88),[68] the 10β-configuration for the natural cyperones [47] is confirmed. The complete stereochemistry (85) of α-cyperone follows from its synthesis from (+)-dihydrocarvone (81).[71] The major product of the synthesis is the epimeric compound (84); conformational principles [72] indicate that the primary condensation product (82) of the Robinson–Mannich reaction

[64] L. Ruzicka and A. G. van Veen, *Annalen*, 1929, **476**, 70.
[65] C. A. Grob and W. Baumann, *Helv. Chim. Acta*, 1955, **38**, 594.
[66] E. Yanovsky, *J. Amer. Chem. Soc.*, 1930, **52**, 3446.
[67] P. D. Gardner and W. J. Horton, *ibid.*, 1955, **77**, 3646.
[68] D. H. R. Barton and E. J. Tarlton, *J.*, 1954, 3492; *Ann. Reports*, 1954, **51**, 206.
[69] W. A. Ayer and W. I. Taylor, *J.*, 1955, 3027.
[70] R. Howe and F. J. McQuillin, *J.*, 1955, 2423.
[71] W. Hückel, *J. prakt. Chem.*, 1941, **157**, 225; N. L. McNiven and J. Read, *J.* 1952, 159; A. J. Birch, *Ann. Reports*, 1950, **47**, 191.
[72] *Ann. Reports*, 1954, **51**, 211.

should be formed most rapidly, and that the thermodynamically more stable product (83) probably arises from a slow equilibration reaction. The two other stereoisomers (86) and (87) have been made from (—)-dihydrocarvone.[73] Isomerisation of each of the four stereoisomers with sulphuric acid affords either β-cyperone (88) or its optical antipode (89).

Autoxidation of α-cyperone in alkaline solution gives a hydroxy-compound [74] formulated as (91) instead of the expected isomer (90) [75] because of failure to oxidise it with manganese dioxide.

Iresin. This extractive from the Mexican shrub, *Iresin celosiodes*, is believed to have the structure (92),[76, 77] thus being the first known sesquiterpene having the bicyclic system characteristic of many diterpenes and triterpenes. Dehydrogenation of iresin (92) yields a mixture of 1 : 5-di-



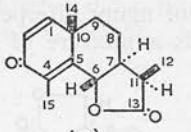
methylnaphthalene and its 2-hydroxy-compound. Oxidation of the triphenylmethyl derivative of (92) gives the ketone (93; R = CPh_3) which on hydrolysis undergoes a retro-aldol reaction giving formaldehyde and the ketone (94), thus revealing the relative position of the two hydroxyl groups in iresin. Ozonolysis of the *OO*-ethylidene derivative of iresin gives the enol lactone (95) which cyclises in presence of acid to the internal acetal (96) whose formation requires that the substituents at positions 3, 4, and 5 be *cis* with respect to each other.

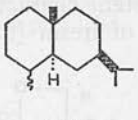
Santonin.[78] Evidence from molecular rotations [79] supports the absolute configuration (97) already advanced [78] for the naturally occurring α-santonin. It has been suggested [80] that α-santonin be named simply santonin, and that the other fifteen stereoisomers should be named by prefixing the steric differences from santonin. The parent hydrocarbon can be conveniently named eudesmane, with the stereochemistry shown in (98). The synthesis

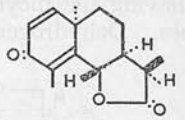
[73] F. J. McQuillin, *J.*, 1955, 528.
[74] H. M. E. Cardwell and F. J. McQuillin, *J.*, 1955, 525.
[75] Cf. E. G. E. Hawkins, *J.*, 1955, 3288.
[76] C. Djerassi, P. Sengupta, J. Herran, and F. Walls, *J. Amer. Chem. Soc.*, 1954, **76**, 2966.
[77] C. Djerassi, W. Rittel, A. L. Nussbaum, F. W. Donovan, and J. Herran, *ibid.*, p. 6410.
[78] *Ann. Reports*, 1954, **51**, 208.
[79] E. J. Corey, *J. Amer. Chem. Soc.*, 1955, **77**, 1044.
[80] W. Cocker and R. S. Cahn, *Chem. and Ind.*, 1955, 384.

of the two hitherto unknown santonins [(99) and its 11-epimer] has been claimed, so far without supporting evidence.[81]

The *cis*-fused lactones (±)- and (−)-12-norsantonin have been synthesised.[82, 83] It is claimed [84] that *cis*-fused lactones of the santonin type undergo reduction with zinc and acetic acid to the deoxy-acids (100; R′ = H; R = R″ = M), whilst the *trans*-lactones are unaffected. Tetrahydrosantonin has been oxidised to the corresponding keto-acid (100; R = R″ = Me; R′ = :O; with double bonds reduced) by use of chromium trioxide in pyridine.[85]


(97)


(98)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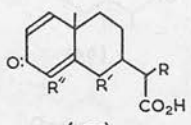
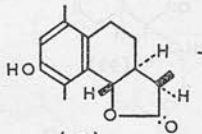
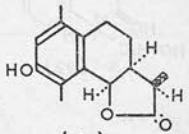
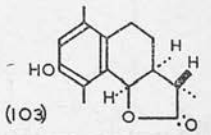

(100)


(101)


(102)

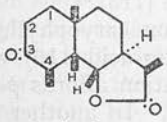

(103)

The acetates of the hitherto unknown desmotroposantonins [(101) and its 11-epimer], having a *trans*-fused lactone ring, have been obtained from santonin (97) and 11β(H)-santonin.[86] Both are readily converted into the more stable *cis*-lactones [(102) and its 11-epimer]. However, while with the *cis*-lactone (102) the action of potassium carbonate in boiling xylene leads to an epimerisation at $C_{(11)}$ to give (103), which would be expected on general conformational principles to be the more stable arrangement,[87] with the *trans*-fused lactone system of santonin the same procedure leads to the epimerisation of 11β(H)-santonin to santonin (97).[86] This reversal of the expected order of stabilities may be due to interference between the 11-methyl group and the carbonyl-oxygen atom in the *trans*-lactone (cf. ref. 38).

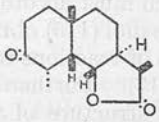
Three tetrahydrosantonins, namely, the so-called α (104), β (105), and γ (106), have been prepared.[88] It is claimed that the β-isomer previously

[81] Y. Abe, T. Harukawa, H. Ishikawa, T. Miki, and M. Sumi, *Chem. and Ind.*, 1955, 91.
[82] T. Miki, *J. Pharm. Soc. Japan*, 1955, **75**, 399.
[83] F. D. Gunstone and A. P. Tulloch, *J.*, 1955, 1130.
[84] T. Miki, *J. Pharm. Soc. Japan*, 1955, **75**, 410, 412.
[85] H. Matsumura, I. Iwai, and E. Ohki, *ibid.*, 1954, **74**, 1029, 1206.
[86] W. Cocker and T. B. H. McMurry, *J.*, 1954, 4430.
[87] N. M. Chopra, W. Cocker, and J. T. Edward, *Chem. and Ind.*, 1955, 41.
[88] M. Yanagita and A. Tahara, *J. Org. Chem.*, 1955, **20**, 959.

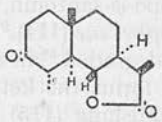
described is a mixture.[89] The α- and the γ-form are stable to acid, whilst the β-form is converted by acid into the α-form. Hence the latter differs



(104)

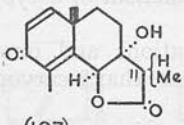


(105)

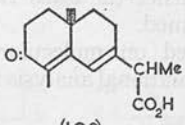


(106)

from the β-form only at $C_{(4)}$; the α- and the γ-form have equatorial methyl at $C_{(4)}$. The α- (and β-)form is considered to have *cis*-fused A/B rings because it gives a 2 : 4-dibromo-derivative, whilst the γ-form (*trans*-fused) gives a 2 : 2-dibromo-compound which rearranges to the 2 : 4-isomer when kept. Similar properties are shown by 3-keto-steroids with *cis*- and *trans*-fused rings respectively.

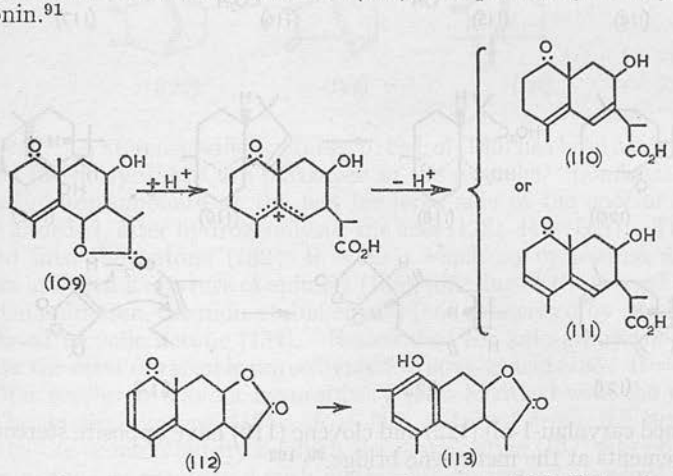


(107)



(108)

Artemisin. Two racemic artemisins (107) epimeric at $C_{(11)}$,[90] and differing from the naturally occurring sesquiterpene in having *cis*-fused lactones,[79] have been made from an intermediate (108) used previously in a synthesis of santonin.[91]



(109) (110) (111)

(112) (113)

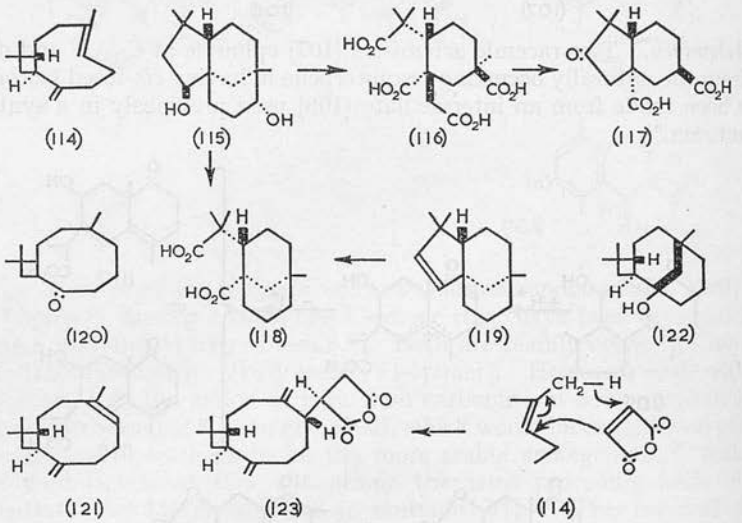
ψ-Santonin. The structure of this sesquiterpene (109)[92] has been confirmed.[93] ψ-Santonic acid, the primary product of acid-catalysed rearrangement, is either (110) or (111); evidence has been advanced in favour of

[89] Cf. (Sir) John Simonsen and D. H. R. Barton, "The Terpenes," Cambridge Univ. Press, 1952, Vol. III, p. 256.
[90] Y. Abe and M. Sumi, *Proc. Japan Acad.*, 1955, **31**, 309.
[91] *Ann. Reports*, 1954, **51**, 208.
[92] N. M. Chopra, W. Cocker, B. E. Cross, J. T. Edward, D. H. Hayes, and H. P. Hutchison, *J.*, 1955, 588; *Ann. Reports*, 1954, **51**, 212.
[93] W. G. Dauben and P. D. Hance, *J. Amer. Chem. Soc.*, 1955, **77**, 606.

each structure.[94, 95] This compound affords anhydro-ψ-santonic acid (112) when heated to 200°;[94, 95] the latter on treatment with acid gives (+)-β-desmotropo-ψ-santonin,[96] which must accordingly be (113).[97]

Caryophyllene (114).[98] The diol (115) obtained from caryophyllene oxide has been degraded [99] in a series of reactions to the tetra-acid (116), which on pyrolysis forms the ketone (117). Further degradation affords *p*-cymene, thus establishing (115) as the structure of the diol. In another series of reactions the diol has been degraded to clovenic acid (118), thus proving the structure of clovene (119). *iso*Caryophyllene (121), when treated with monoperphthalic acid, affords two oxides; both these and the oxide of caryophyllene may be converted into the same ketone (120) and so are stereoisomers.[100] Models indicate that while two oxides may be formed by addition of oxygen to either side of the endocyclic *cis*-double bond of (121), only the oxide formed by addition from above to the *trans*-double bond of (114) is possible; hence the *trans–cis*-isomerism of caryophyllene–*iso*caryophyllene [99] is confirmed.

Arguments based on molecular rotations and on interpretations of reactions by conformational analysis indicate that β-caryophyllene alcohol [101]

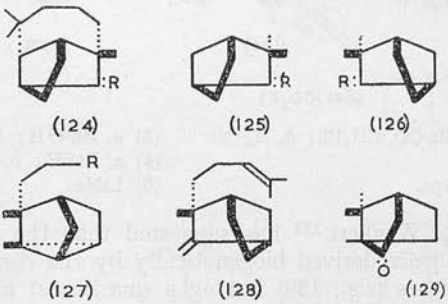


(renamed caryolan-1-ol) (122) and clovene (119) have opposite stereochemical arrangements at the methylene bridge.[99, 102]

[94] W. Cocker, *Chem. and Ind.*, 1955, 1040.
[95] W. G. Dauben, P. D. Hance, and W. K. Hayes, *J. Amer. Chem. Soc.*, 1955, **77**, 4609.
[96] W. Cocker, B. E. Cross, and C. Lipman, *J.*, 1949, 959.
[97] Cf. G. R. Clemo and W. Cocker, *J.*, 1946, 30.
[98] *Ann. Reports*, 1954, **51**, 215.
[99] A. Aebi, D. H. R. Barton, A. W. Burgstahler, and A. S. Lindsey, *J.*, 1954, 4659; cf. D. H. R. Barton and A. S. Lindsey, *J.*, 1951, 2988; D. H. R. Barton, T. Bruun, and A. S. Lindsey, *J.*, 1952, 2210; A. Aebi, D. H. R. Barton, and A. S. Lindsey, *J.*, 1953, 3124.
[100] G. R. Ramage and L. Whitehead, *J.*, 1954, 4336.
[101] Cf. J. M. Robertson and G. Todd, *Chem. and Ind.*, 1953, 437.
[102] D. H. R. Barton and A. Nickon, *J.*, 1954, 4665.

The maleic anhydride adduct of caryophyllene has most probably the formula (123), arising by reaction at the allylic position according to the mechanism shown.[103]

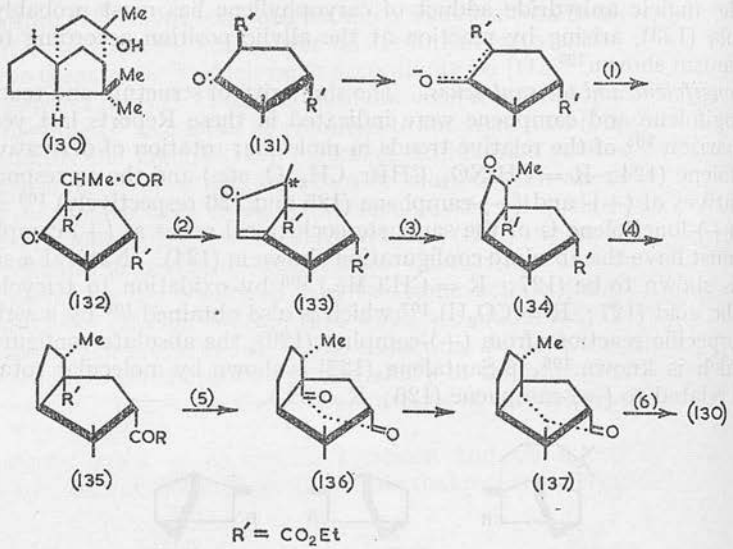
Longifolene and the santalenes. The similarity of structure and reactions of longifolene and camphene were indicated in these Reports last year.[104] Comparison [105] of the relative trends in molecular rotation of derivatives of longifolene (124; R = CH·NO_2, CHBr, CH_2, O, etc.) and the corresponding derivatives of (+)- and (−)-camphene (125 and 126 respectively) [106] shows that (+)-longifolene is of the same stereochemical series as (+)-camphene, and must have the absolute configuration shown in (124). Natural α-santalene is shown to be (127; R = CH:CMe_2) [105] by oxidation to tricyclo*eka*-santalic acid (127; R = CO_2H),[107] which is also obtained [108] by a series of stereospecific reactions from (+)-camphor (129), the absolute configuration of which is known.[106] β-Santalene (128) is shown by molecular rotations to be related to (−)-camphene (126; R = H_2).



(124) (125) (126) (127) (128) (129)

Cedrol.[109] A stereospecific synthesis of cedrol (130) has been achieved;[110] the stages in the synthesis are portrayed in the formulæ. Condensation of benzyl α-bromopropionate at the less hindered side of the enol of ketone (131) [111] afforded, after hydrogenolysis, the acid (132; R = OH). This was converted into the ketone (132; R = Me), which on cyclisation and dehydration afforded a mixture of epimers (133), differing at the starred carbon atom. On reduction, the more stable epimer (not epimerised by alkali) gave the *cis*-fused bicyclic ketone (134). Removal of the keto-group *via* a thioketal gave the ester of racemic norcedrenedicarboxylic acid (135; R = OEt), which after resolution gave a lævorotatory form identical with the natural acid. The methyl ketone (135; R = Me) obtained from the acid was cyclised to the dione (136) from which the monoketone (137) was obtained. Reaction of the last compound with methyl-lithium gave (−)-cedrol (130).

[103] A. Nickon, *J. Amer. Chem. Soc.*, 1955, **77**, 1190.
[104] *Ann. Reports*, 1954, **51**, 215.
[105] G. Ourisson, *Bull. Soc. chim. France*, 1955, 895.
[106] Cf. A. J. Birch, *Ann. Reports*, 1950, **47**, 192.
[107] F. W. Semmler and K. Bode, *Ber.*, 1907, **40**, 1124; cf. S. C. Bhattacharyya, *Sci. and Cult.*, 1943, **13**, 158.
[108] P. C. Guha and S. C. Bhattacharyya, *J. Indian Chem. Soc.*, 1944, **21**, 261.
[109] *Ann. Reports*, 1954, **51**, 216.
[110] G. Stork and F. H. Clarke, *J. Amer. Chem. Soc.*, 1955, **77**, 1072.
[111] C. S. Gibson, K. V. Hariharan, and J. L. Simonsen, *J.*, 1927, 3009.



R′ = CO_2Et

(1) *a*, Br·CHMe·CO_2·CH_2Ph; *b*, H_2–Ni.
(2) *a*, Bu^tOK; *b*, C_7H_7·SO_3H.
(3) H_2–Pd.
(4) *a*, EtSH; *b*, H_2–Ni.
(5) several steps.
(6) LiMe.

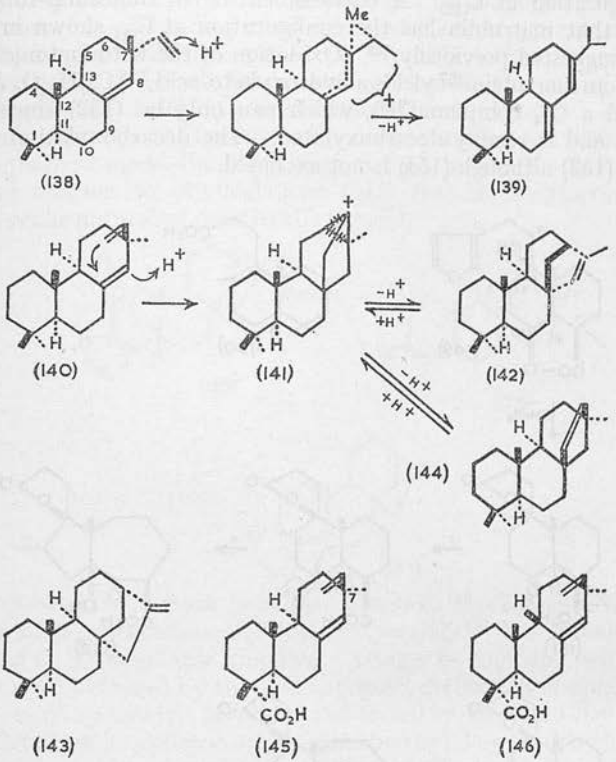
Diterpenes. E. Wenkert [112] has suggested that the abietane-type di terpenes (*e.g.*, 139) are derived biogenetically by the concerted rearrang ment of pimaradienes (*e.g.*, 138) having a quasi-axial methyl and qua equatorial vinyl function. He suggests that this rearrangement takes pla so readily that only pimaradienes (and diterpenes derived from the epimeric at $C_{(7)}$ to (138) [*e.g.*, (140)] will be found in Nature. Thus rimuene is formulated as (140); its isomerisation to *iso*phyllocladene (142) probab involves the non-classical carbonium ion (141) and reveals the possi biogenesis and stereochemistry of phyllocladene (143).[114] The structu (144) is suggested for the isomeric tetracyclic diterpene mirene,[115] which also converted into *iso*phyllocladene by acid, probably *via* the same int mediate (141). The structural and biogenetic relations of (142) and (14 to veatchine and atisine are obvious (see Alkaloids section).

*iso*Dextropimaric acid is generally formulated [116] as a 7-epimer of dext pimaric acid (145); in accordance with the rule above, Wenkert suggests th instead it is (146), epimeric at $C_{(13)}$. This is compatible with all availa experimental evidence.

Cassaic acid. This αβ-unsaturated hydroxyketo-acid is obtained hydrolysis of cassaine, an *Erythrophleum* alkaloid.[117] It can be reduced several stages to a saturated hydroxy-acid, cassanic acid. Methyl cassan

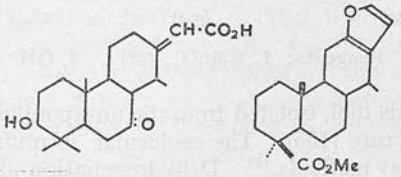
[112] E. Wenkert, *Chem. and Ind.*, 1955, 282; cf. L. Ruzicka, *Experientia*, 1953, 9,
[113] C. W. Brandt, *New Zealand J. Sci. Technol.*, 1928, **20**, 8.
[114] Cf. W. Bottomley, A. R. H. Cole, and D. E. White, *J.*, 1955, 2624.
[115] J. R. Hosking and W. F. Short, *Rec. Trav. chim.*, 1928, **47**, 834.
[116] G. C. Harris and T. F. Sanderson, *J. Amer. Chem. Soc.*, 1948, **70**, 2081.
[117] G. Dalma, *Helv. Chim. Acta*, 1939, **22**, 1947; L. Ruzicka and G. Dalma,
p. 1516.

reaction with methylmagnesium iodide followed by dehydrogenation, rds a hydrocarbon [118] identified as 2-*iso*butyl-1 : 8-dimethylphenanthrene



(138) (139) (140) (141) (142) (144) (143) (145) (146)

synthesis.[119] On the basis of this and other evidence cassaic acid is nulated as (147).

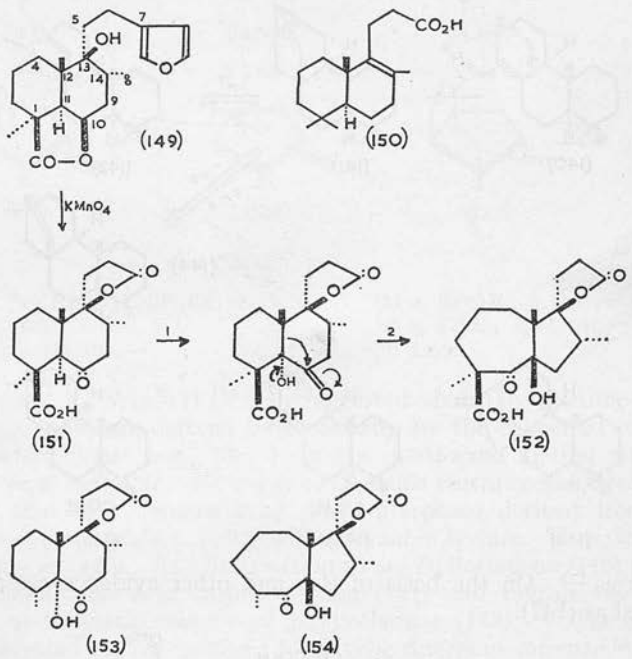


(147) (148)

Methyl vouacapenate. This compound (148), from *Vouacapoua ameri-a* [120] and *V. macropetula*,[121] is the 1-epimer of methyl vinhaticoate,[122] ce their rates of hydrolysis are comparable with those of methyl podo-pate and methyl abietate respectively.

[118] L. Ruzicka, B. G. Engel, A. Ronco, and K. Berse, *Helv. Chim. Acta*, 1945, **28**, 8; B. G. Engel, A. Ronco, K. Berse, P. A. Plattner, and L. Ruzicka, *ibid.*, 1949, 1713.
[119] L. G. Humber and W. I. Taylor, *J.*, 1955, 1044.
[120] D. B. Spoelstra, *Rec. Trav. chim.*, 1930, **49**, 226.
[121] F. E. King, D. H. Godson, and T. J. King, *J.*, 1955, 1117.
[122] F. E. King, T. J. King, and K. G. Neill, *J.*, 1953, 1055.

Marrubiin. This lactone (149) [123] has been related [124] to ambrein by conversion into the degradation product (150) [125] of the latter. This proves the configuration at $C_{(12)}$. A reassessment [126] of molecular-rotation data indicates that marrubiin has the configuration at $C_{(1)}$ shown in (149) and not that suggested previously.[123] Oxidation of the keto-lactonic acid (151) derived from marrubiin [127] yields a hydroxyketo-acid,[128] $C_{17}H_{24}O_6$ (previously considered a C_{14} compound [129]), which can only be (152) since it readily lactonises and is readily decarboxylated. The decarboxylation product is probably (153) although (154) is not excluded.

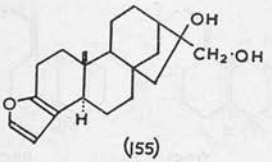


Reagents: 1, $KMnO_4$; OH^-. 2, OH^-.

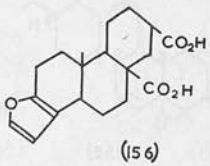
Cafestol.[130] This diol, isolated from the unsaponifiable part of coffee oil, probably has structure (155). The molecular formula $C_{20}H_{28}O_3$ has been established by *X*-ray methods.[131] Dehydrogenation of the derivative (15

[123] *Ann. Reports*, 1954, **51**, 217.
[124] D. Burn and W. Rigby, *Chem. and Ind.*, 1955, 386.
[125] L. Ruzicka and F. Lardon, *Helv. Chim. Acta*, 1946, **29**, 912; P. Dietrich and Lederer, *ibid.*, 1952, **35**, 1148; P. Dietrich, E. Lederer, and D. Mercier, *ibid.*, 1954, 705.
[126] W. Cocker, J. T. Edward, and T. F. Holley, *Chem. and Ind.*, 1955, 772.
[127] W. Cocker, B. E. Cross, S. R. Duff, J. T. Edward, and T. F. Holley, *J.*, 19 2540.
[128] W. Cocker, J. T. Edward, T. F. Holley, and D. M. S. Wheeler, *Chem. and I* 1955, 1484.
[129] E. Ghigi, *Gazzetta*, 1948, **78**, 865; 1951, **81**, 336; 1953, **83**, 252.
[130] *Ann. Reports*, 1953, **50**, 210.
[131] R. D. Haworth, A. H. Jubb, and J. McKenna, *J.*, 1955, 1983.

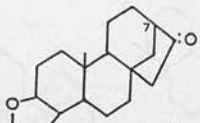
gives 2-hydroxyphenanthrene and 1-ethyl-2-hydroxyphenanthrene,[132, 133] the position of the furan [134] oxygen atom thus being shown. The >C(OH)·CH_2·OH group may be degraded to a carbonyl group which is in a *cyclo*pentane ring. This ring in epoxynorcafestanone appears to be fused to the perhydrophenanthrene system as in (157); thus the monobromo-derivative cannot be dehydrobrominated, as would be expected if the bromine were at $C_{(7)}$ (Bredt's rule). Cafestol has one *C*-methyl group. The structure (155) would represent the first naturally occurring diterpene with an ethenoid group in place of a *gem*-dimethyl group at $C_{(1)}$; biogenetically it would bear the same relation to phyllocladene (143) that methylabietin [135] (which does not occur naturally) does to abietic acid.



(155)



(156)



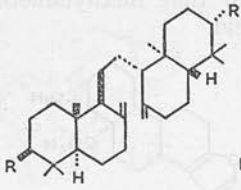
(157)

Triterpenes.—α-Onocerin (α-onoceradienediol).[136] The elucidation of the structure of α-onoceradienediol (158; R = OH) [137] has revealed the first squalenoid of the vegetable kingdom. It may be compared with *tetracyclo*squalene (162) obtained by the acid-catalysed cyclisation of squalene,[138] and is unique in its symmetry, involving tail-to-tail union of two *dicyclo*farnesene units. Details of its skeleton are revealed by the following dehydrogenations. α-Onoceradiene (158; R = H) [136] affords 1 : 2 : 5-trimethylnaphthalene, and the diol itself (158; R = OH) gives 1 : 2 : 5 : 6-tetramethylnaphthalene. Ozonolysis of the diol diacetate (158; R = OAc) gives two mols. of formaldehyde and the diketone (159; R = OAc) from which dinoronocerane (160) is obtained by conventional methods. On dehydrogenation the hydrocarbon (160) gives 1 : 5-dimethylnaphthalene and this result, with the results of the dehydrogenations already described, locates the position of the $=CH_2$ groups. The symmetry of the molecule and the equivalence of the hydroxyl groups were demonstrated by protecting each hydroxyl group in turn, oxidising the other to a ketone group, and then removing this by the Wolff–Kishner method. The same α-onoceradieneol was obtained by each route.

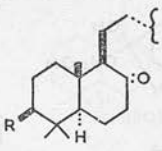
α-Onoceradienediol and its derivatives undergo isomerisation on vigorous

[132] C. Djerassi, H. Bendas, and P. Sengupta, *J. Org. Chem.*, 1955, **20**, 1046.
[133] H. Bendas and C. Djerassi, *Chem. and Ind.*, 1955, 1481.
[134] A. Wettstein, H. Fritzsche, F. Hunziker, and K. Miescher, *Helv. Chim. Acta*, 1941, **24**, 332E; A. Wettstein and K. Miescher, *ibid.*, 1943, **26**, 631; A. Wettstein, F. Hunziker, and K. Miescher, *ibid.*, p. 1197; A. Wettstein, M. Spillmann, and K. Miescher, *ibid.*, 1945, **28**, 1004.
[135] L. Ruzicka, *ibid.*, 1932, **15**, 1300.
[136] Elsevier's "Encyclopædia of Organic Chemistry," Vol. 14, p. 608; Suppl., p. 1313.
[137] D. H. R. Barton and K. H. Overton, *Chem. and Ind.*, 1955, 654; *J.*, 1955, 2639.
[138] L. Ruzicka, *Experientia*, 1953, **9**, 357.

treatment with acid to the pentacyclic γ-series of compounds of structure and configuration (161). The stereochemistry of α-onoceradienediol shown in (158), which is likely to be its absolute configuration, is derived as follows. Reduction of the diketone (158; R = :O) with sodium in propanol regenerates the original diol (158; R = OH) and hence the hydroxyl groups are equatorially bound. Molecular-rotation data are consistent with a 3β-configuration, so that the rings are *trans*-fused.[139] The diketone (159; R = OH) is unchanged by alkali and thus the attachment of the connecting bridge between the two cyclic units must have the β-(equatorial) configuration.



(158)



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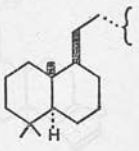
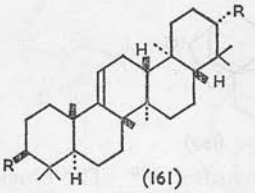
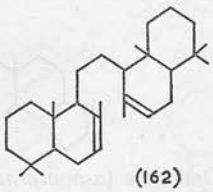


(160)



(161)



(162)

α-Amyrin. The conversion of a derivative of α-amyrin into an oleanane derivative containing all five rings has been accomplished for the first time.[140] α-Amyrin acetate (163; R = Ac) is converted by selenium dioxide into the diene (164), which on treatment with hydrochloric–acetic acid gives oleana-11 : 13(18)-dienyl acetate (165). This establishes the stereochemistry shown in (163) for α-amyrin. A β(H)-configuration at $C_{(18)}$ is made probable by a study of the αβ-unsaturated ketone (166) derived from α-amyrin.[141] This is oxidised, with rearrangement, by selenium dioxide in acetic acid to afford the dienone (167). A similar oxidation with rearrangement takes place with the analogous ketone derived from β-amyrin having a β-hydrogen atom at $C_{(18)}$; the ketone having an α-hydrogen atom at $C_{(18)}$ is unaffected.

Further evidence that the configurations at $C_{(17)}$ and $C_{(18)}$ are similar in the oleanane and the ursane series comes from a comparison of the optical rotations and stabilities of the lactones of oleanolic and ursolic acids. However, in the ursane series, *cis*-D/E ring fusion is the thermodynamically stable arrangement, as shown by the facts that 12-en-11-ones are not isomerised [142] and that the monoene (168), obtained by hydrogenation of the diene (164), is rearranged by acid into α-amyrin and not its 18-epimer.[140]

The stable *cis*-D/E ring fusion indicates that ring E is probably a five-

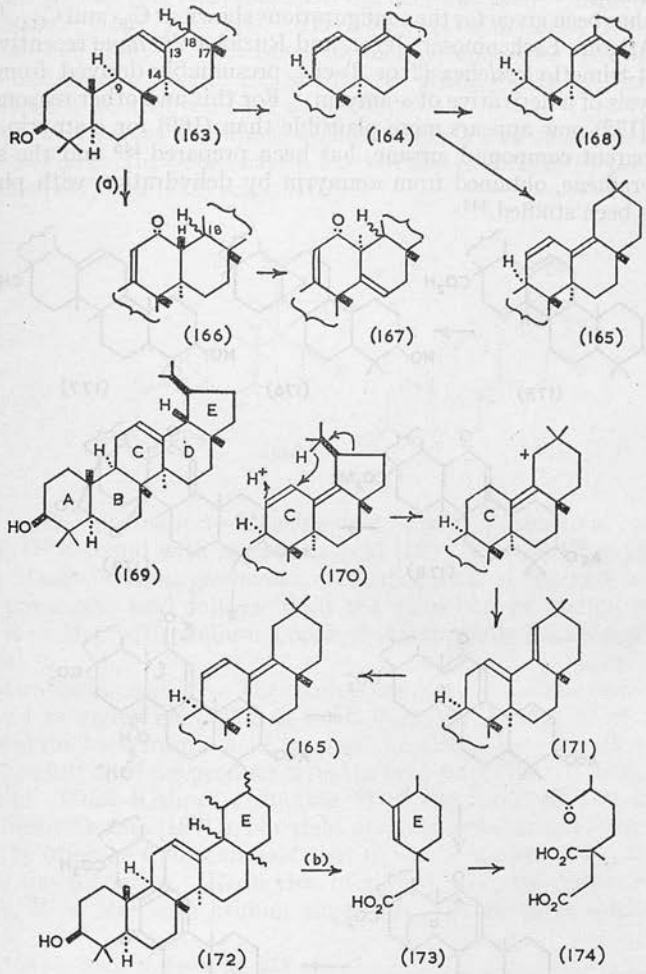
[139] D. H. R. Barton and E. R. H. Jones, *J.*, 1944, 659; W. Klyne and W. M. Stokes, *J.*, 1954, 1979; D. H. R. Barton, *Experientia*, 1950, **6**, 316.

[140] G. G. Allan, J. M. Beaton, J. I. Shaw, F. S. Spring, R. Stevenson, J. L. Stewart, and W. S. Strachan, *Chem. and Ind.*, 1955, 281; J. M. Beaton, F. S. Spring, R. Stevenson, and W. S. Strachan, *J.*, 1955, 2610.

[141] G. G. Allan and F. S. Spring, *J.*, 1955, 2125.

[142] E. J. Corey and J. J. Ursprung, *Chem. and Ind.*, 1954, 1387.

membered ring (although a six-membered ring is not excluded)[142] and F. S. Spring and his colleagues[140] have advanced the formula (169) for α-amyrin. The *iso*propyl group is given the β-orientation to explain the severe hindrance to the double bond; its presence is supported by infrared



(*a*) Several steps. (*b*) Eleven steps.

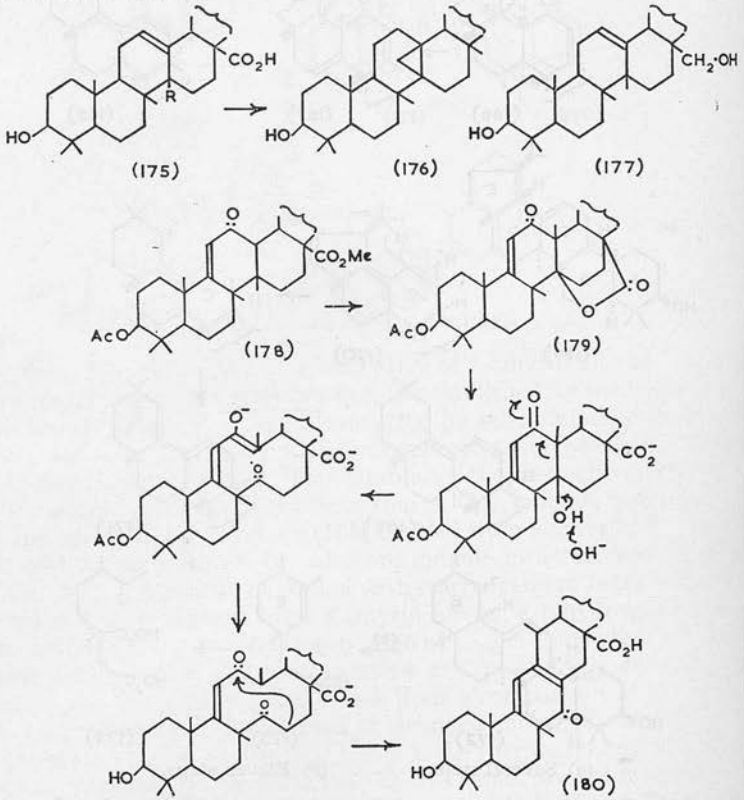
evidence.[143] The rearrangement of the ursadiene (164) into the oleanadiene (165) is explained by the plausible mechanism (170) ⟶ (171) ⟶ (165).

The cyclic fragment (173) obtained by an eleven-stage degradation of α-amyrin[144] and derived from the original ring E, has been further studied.[145] It is ozonised to a keto-dicarboxylic acid (174), in which the presence of an

[143] G. D. Meakins, *Chem. and Ind.*, 1955, 1353.
[144] O. Jeger, R. Rüegg, and L. Ruzicka, *Helv. Chim. Acta*, 1947, **30**, 1294; cf. *Ann. Reports*, 1950, **47**, 200.
[145] A. Meisels, R. Rüegg, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, 1955, **38**, 1298.

acetyl group has been established. However, this and previous evidence for the formula (172) depend on pyrolytic reactions in which the possibil of rearrangement is not excluded. For the moment the formulæ of t family of compounds based on α-amyrin must be regarded as unsettl and accordingly we employ the non-committal formulation (163). Additio evidence has been given for the configurations shown at $C_{(9)}$ and $C_{(14)}$.[140, 142] Melera, Arigoni, Eschenmoser, Jeger, and Ruzicka [145a] have recently isola a 1 : 2 : 4-trimethyl*cyclo*hex-(2 or 3)-ene, presumably derived from ring by pyrolysis of a derivative of α-amyrin. For this and other reasons,[145b] formula (172) now appears more plausible than (169) for α-amyrin.

The parent compound, ursane, has been prepared,[146] and the struct of α-amyradiene, obtained from α-amyrin by dehydration with phosph oxide has been studied.[147]



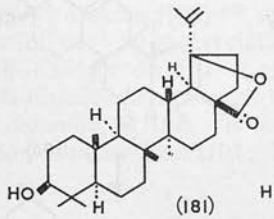
Triterpenes related to α-Amyrin.—It has been confirmed [148] that quino acid (175; R = CO_2H) is of the α-amyrin–ursolic acid type by its conversi

[145a] A. Malera, D. Arigoni, A. Eschenmoser, O. Jeger, and L. Ruzicka, *Helv. Ch Acta*, 1956, in the press.
[145b] E. J. Corey and J. J. Ursprung, *J. Amer. Chem. Soc.*, 1956, **78**, 183.
[146] T. Lyssy and O. Jeger, *Helv. Chim. Acta*, 1955, **38**, 1294.
[147] J. L. Beton and T. G. Halsall, *Chem. and Ind.*, 1954, 1560.
[148] A. Zürcher, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, 1954. **37**, 2145.

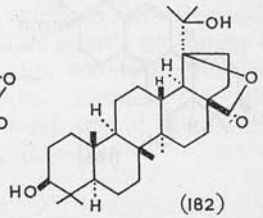
in a series of unambiguous reactions, into phyllanthol (176).[149] This evidence and the conversion of quinovic acid into uvaol (177) fix the position of the hydroxymethyl group in the latter compound. Another partial synthesis of phyllanthol from α-amyrin has been published.[150]

An interesting ring rearrangement has been observed [151] with a derivative of ursolic acid (175; R = Me). The lactone (179) obtained by oxidation of the keto-ester (178) [152] with selenium dioxide undergoes rearrangement in rings C and D on energetic treatment with alkali, giving the dienone (180) (cf. the rearrangement recorded on p. 198).

Triterpenes related to Lupeol.—Thurberogenin and stellatogenin, extractives from the cacti *Lemaireocereus thurberi* and *L. stellatus*,[153] probably have structures (181) and (182) respectively.[154]



(181)



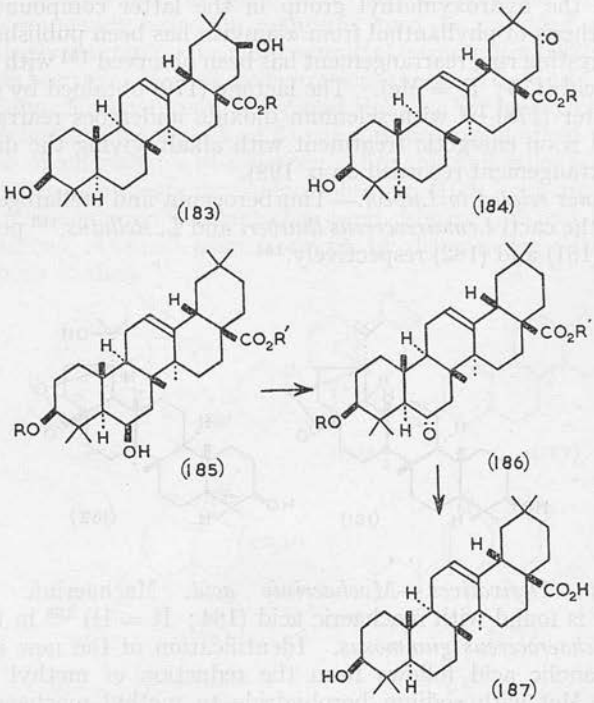
(182)

β-*Amyrin Derivatives.*—*Machaerinic acid.* Machaerinic acid (183; R = H) [155] is found with machaeric acid (184; R = H) [156] in the Mexican cactus, *Machaerocereus gummosus*. Identification of the new acid as 21β-hydroxyoleanolic acid follows from the reduction of methyl machaerate (184; R = Me) with sodium borohydride to methyl machaerinate (183; R = Me).

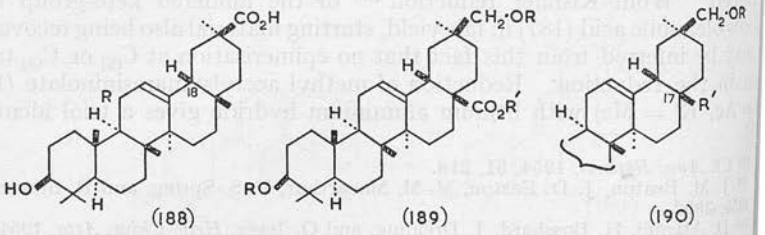
Sumaresinolic acid.[157] The stereochemistry of sumaresinolic acid is portrayed in structure (185; R = R′ = H).[158] Oxidation of its methyl ester acetate by chromic acid in pyridine (185; R = Ac, R′ = Me) and partial hydrolysis of the product gives the keto-ester (186; R = H, R′ = Me). "Forced" Wolff–Kishner reduction [159] of the hindered keto-group then affords oleanolic acid (187) in fair yield, starting material also being recovered. It may be inferred from this fact that no epimerisation at $C_{(3)}$ or $C_{(5)}$ takes place in the reduction. Reduction of methyl acetylsumaresininolate (185; R = Ac, R′ = Me) with lithium aluminium hydride gives a triol identical

[149] Cf. *Ann. Reports*, 1954, **51**, 218.
[150] J. M. Beaton, J. D. Easton, M. M. Macarthur, F. S. Spring, and R. Stevenson, *J.*, 1955, 3992.
[151] D. Arigoni, H. Bosshard, J. Dreiding, and O. Jeger, *Helv. Chim. Acta*, 1954, **37**, 2173.
[152] J. Dreiding, O. Jeger, and L. Ruzicka, *ibid.*, 1950, **33**, 1325.
[153] C. Djerassi, L. E. Geller, and A. J. Lemin, *J. Amer. Chem. Soc.*, 1953, **75**, 2254.
[154] C. Djerassi, E. Farkas, L. H. Liu, and G. H. Thomas, *ibid.*, 1955, **77**, 5330.
[155] C. Djerassi and A. E. Lippman, *ibid.*, p. 1825.
[156] *Ann. Reports*, 1954, **51**, 220.
[157] L. Ruzicka, A. Grob, and H. Hösli, *Helv. Chim. Acta*, 1943, **26**, 2283; L. Ruzicka, J. Norymberski, and O. Jeger, *ibid.*, 1945, **28**, 380.
[158] C. Djerassi, G. H. Thomas, and O. Jeger, *ibid.*, 1955, **38**, 1304.
[159] D. H. R. Barton, D. A. J. Ives, and B. R. Thomas, *J.*, 1955, 2056.

with that obtained from the ester acetate (186; R = Ac, R′ = Me) ar
hence the 6-hydroxyl group is β (axial).[160]



Glycyrrhetic acid. The configuration of this acid, apart from the co
figuration of the carboxyl group, has been disclosed by its conversion
into β-amyrin.[162] Methyl glycyrrhetate has recently been converted into
18-epimer by treatment with acid, and with strong alkali.[163] Comparis
of the rates of hydrolysis of the two esters shows that methyl glycyrrheta



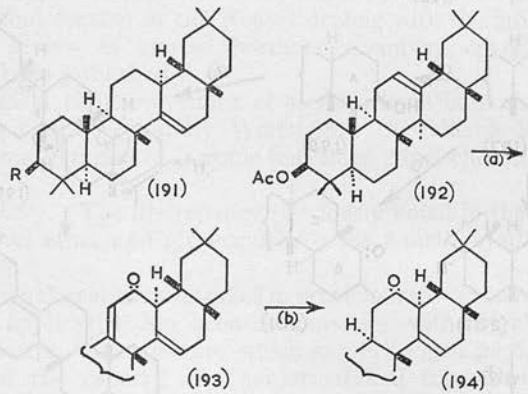
has the more hindered methoxycarbonyl group. If a chair conformation
assumed for ring E, glycyrrhetic acid thus becomes (188; R = :O).

Queretaroic acid. The Mexican cacti, *Lemaireocereus queretaroensis* a

[160] D. H. R. Barton, *J.*, 1953, 1027.
[161] L. Ruzicka and A. Marxer, *Helv. Chim. Acta*, 1939, **22**, 195; cf. L. Ruzi
O. Jeger, and W. Ingold, *ibid.*, 1943, **26**, 2278.
[162] D. H. R. Barton and N. J. Holness, *J.*, 1952, 78.
[163] J. M. Beaton and F. S. Spring, *J.*, 1955, 3126.

L. beneckei, contain a new triterpene acid, which is 30-hydroxyoleanolic acid (189; R = R′ = H).[164] Oxidation of its methyl ester and reduction of the resulting keto-aldehyde by the Wolff–Kishner method gives methyl 3-deoxo-oleanolate, and the pentacyclic skeleton and position of the carboxyl group are thus established. Rosenmund reduction of the diacetyl derivative of queretaroyl chloride gives the 17-aldehyde diacetate (190; R = Ac, R′ = CHO) which is readily converted into olean-12-ene-3(β) : 30-diol (190; R = H, R′ = Me). The last product is identical with the diol obtained by the reduction of 11-deoxoglycyrrhetic acid (188; R = H_2) with lithium aluminium hydride.

Taraxerol.[165] The identity of taraxerol (191; R = OH) with skimmiol[166] has been proved by direct comparison.[167] A partial synthesis of taraxerol from β-amyrin acetate (192) has been achieved.[168] β-Amyrin acetate is converted by established methods into 12-oxo*iso*olean-9(11) : 14-dien-3β-yl acetate (193),[169] which on reduction with lithium and liquid ammonia followed by reacetylation affords the ketone (194). Under "forced" Wolff–Kishner conditions this ketone is reduced to taraxerol (191; R = OH) identified as its acetate. Taraxerene, recently isolated[170] from *Cladonia deformis*, is (191; R = H), and is obtained by the Wolff–Kishner reduction of taraxerone (191; R = :O).[167]

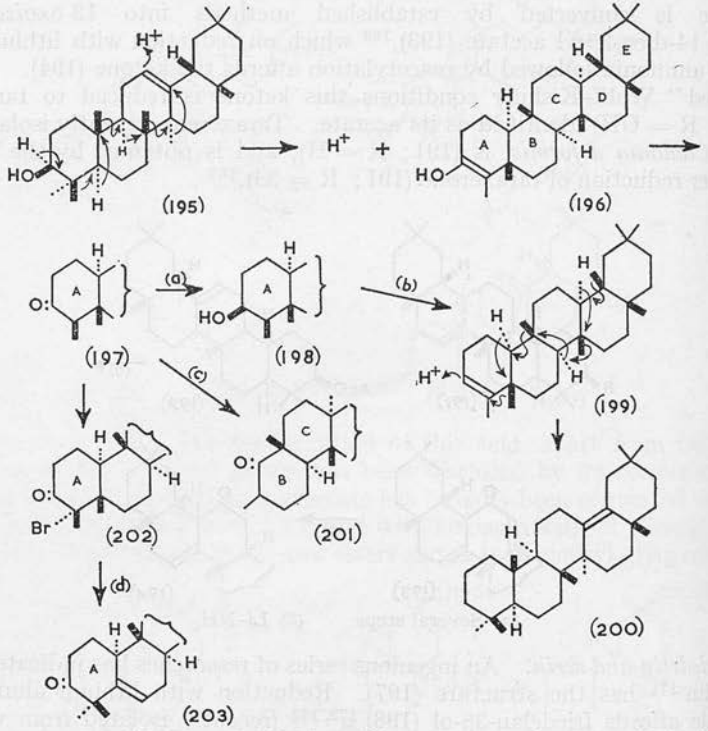


(*a*) Several steps. (*b*) Li–NH_3.

Friedelin and cerin. An ingenious series of researches has indicated that friedelin[171] has the structure (197). Reduction with lithium aluminium hydride affords friedelan-3β-ol (198)[172, 173] (recently isolated from various

[164] C. Djerassi, J. A. Henry, A. J. Lemin, and T. Rios, *Chem. and Ind.*, 1955, 1520.
[165] *Ann. Reports*, 1954, **51**, 222.
[166] K. Takeda, *J. Pharm. Soc. Japan*, 1941, **61**, 117.
[167] C. J. W. Brooks, *J.*, 1955, 1675.
[168] J. M. Beaton, F. S. Spring, R. Stevenson, and J. L. Stewart, *Chem. and Ind.*, 1955, 35; *J.*, 1955, 2131; C. J. W. Brooks, *ibid.*, p. 1675.
[169] Cf. G. G. Allan, J. D. Johnston, and F. S. Spring, *J.*, 1954, 1546; J. D. Johnston and F. S. Spring, *ibid.*, p. 1556.
[170] T. Bruun, *Acta Chem. Scand.*, 1954, **8**, 1291.
[171] Elsevier's "Encyclopædia of Organic Chemistry," Vol. 14, pp. 588, 1182.
[172] E. J. Corey and J. J. Ursprung, *J. Amer. Chem. Soc.*, 1955, **77**, 3667, 3668.
[173] G. Brownlie, F. S. Spring, R. Stevenson, and W. S. Strachan, *Chem. and Ind.*, 1955, 686, 1156.

natural sources).[174] This is converted by phosphorus oxychloride in pyridine into friedel-3-ene (199). Treatment of the compound (198) or (199),[173] or of friedel-2-ene [175] with strong acid results in a concerted rearrangement, giving olean-13(18)-ene (200). The structure (197) thus indicated for friedelin has been supported by an extension of earlier degradative work.[172, 173, 176] The removal of ring A by several stages affords the ketone (201), shown by bromination [176] and deuterium-exchange [172] studies to have only one enolisable hydrogen atom, as required by the formulation shown. The assumption of the presence of a $CH_2 \cdot CH_2 \cdot CO \cdot CH \cdot CH$ group would lead to a ketone having three exchangeable hydrogen atoms. Further this group should, on bromination and dehydrobromination, give an $\alpha\beta$-unsaturated ketone; it is found that 4-bromofriedelin (202), on treatment



Reagents: (*a*), $LiAlH_4$. (*b*), POCl–Pyridine. (*c*), several steps. (*d*), AgOAc.

with silver acetate, undergoes rearrangement as well as dehydrobromination to give the unsaturated ketone (203) which cannot be isomerised to an $\alpha\beta$-unsaturated ketone. A re-interpretation [173] of earlier degradative work [171] has removed the need to postulate the $CH_2 \cdot CH_2 \cdot CO \cdot CH \cdot CH$ group. A large amount of further experimental work [172, 173] supports details of the structure (197).

[174] P. R. Jefferies, *J.*, 1954, 473; T. Bruun, *Acta Chem. Scand.*, 1954, **8**, 71.
[175] H. Dutler, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, 1955, **38**, 1268.
[176] G. Ourisson and T. Takahashi, *Chem. and Ind.*, 1955, 1155.

Cerin [171] is friedelin-2β-ol.[172]

It is suggested [172, 175] that friedelin arises biogenetically from β-amyrin (195) by a concerted rearrangement, operating in the reverse direction to the rearrangement (198) ⟶ (200), which affords the enolic form (196) of friedelin.

W. C.
J. T. E.

7. STEROIDS.

General.[1]—The first part of the steroid supplement to Elsevier's "Encyclopædia," [2a] covering literature on steroid hydrocarbons and monohydroxy-compounds from 1937 to 1946 (with many later references regarding structures up to 1954), appeared during the past year. The first volume of a German monograph [2b] on steroids also appeared.

One of the principal achievements of the year has been the total synthesis of aldosterone. Important progress has been made in the stereochemistry of the trimethyl-steroids (euphol and tirucallol).

Papers on partial synthesis of cortisone decreased in number; interest has turned to modified structures, particularly those carrying 9α-halogen, 1 : 4-dien-3-one, and, most recently, 2-methyl groupings; a table of modified hormones is given on p. 209; the compounds are discussed in detail, where necessary, in that section of the Report dealing with the groups which are modified. A review of steroid hormone chemistry covering the years 1948—53 has been published.[3]

Microbiological transformations of steroids have been surveyed extensively by Fried *et al.*[4a] and by Wettstein.[4b] A valuable review on the partition chromatography of steroids (including paper chromatography) has appeared.[4c]

Stereochemistry. The discrepancy previously noted in the correlation of calciferol methyl ether and glyceraldehyde *via* β-methoxyadipic acid [5] has been cleared up.[6]

Conformational analysis continues to prove its value in steroid chemistry; an important application has been in connexion with neighbouring group reactions.[7] Barton,[8] in a lecture which surveys much unpublished work, has introduced the concept of "conformational transmission," *i.e.*, the transmission of distortions (caused by substituents) through a molecule by flexing of valency angles and alteration of atomic co-ordinates.

[1] Cf. *Ann. Reports*, 1954, **51**, 222.

[2] (*a*) "Elsevier's Encyclopædia of Organic Chemistry," ed. F. Radt, Elsevier, Amsterdam, 1954, Vol. 14, Suppl., pp. 1347—1868 S; (*b*) H. Lettre, R. Tschesche, and H. Fernholz, "Uber Sterine, Gallensäuren and verwandte Naturstoffe," Enke, Stuttgart, 1954, 2nd edn., Vol. I.

[3] H. Hirschmann in "The Hormones," ed. G. Pincus and K. V. Thimann, Academic Press, New York, 1955, Vol. III, p. 521.

[4] (*a*) J. Fried, R. W. Thomas, D. Perlman, J. E. Herz, and A. Borman, *Recent Progr. Hormone Res.*, 1955, **11**, 149; (*b*) A. Wettstein, *Experientia*, 1955, **11**, 465. A shorter account is given by D. H. Peterson, "Perspectives and Horizons in Microbiology," Rutgers Univ. Press, New Brunswick, N.J., p. 121; cf. also J. K. Grant, "Enzymic Hydroxylation of Steroids" (in Biological Chemistry section); (*c*) E. Heftmann, *Chem. Rev.*, 1955, **55**, 679; cf. I. E. Bush, *Brit. Med. Bull.*, 1954, **10**, 229.

[5] A. Lardon and T. Reichstein, *Helv. Chim. Acta*, 1947, **32**, 2003.

[6] M. Viscontini and P. Miglioretto, *ibid.*, 1955, **38**, 930.

[7] G. H. Alt and D. H. R. Barton, *J.*, 1954, 4284.

[8] D. H. R. Barton, *Experientia*, 1955, Suppl. 2, p. 121.

A study of the relative rates of oxidation of steroid secondary alcohols by chromic acid [8a] suggests that, in general, the oxidation occurs the faster, the greater is the decrease in non-bonded interaction energy involved in the transformation of the alcohol into the ketone.

Physical properties. Studies of infrared spectra have dealt with the " finger-print " region for keto-steroids,[9] acetates and methoxy-compounds,[10] 3-hydroxy- and 3-acetoxy-compounds,[11] differences between polymorphic modifications,[12] side-chain ketones,[13] and 20-substituted steroids.[14] Ultra-violet spectra of α-hydroxy-ketones [15] and of αβ-unsaturated ketones (in alkali) [16] have been discussed.

Djerassi and his colleagues [17] have carried out the first extensive studies of optical rotatory dispersion in the steroid field. This work, made possible by the recent introduction of a commercial spectropolarimeter in the United States,[18] promises to open up new fields in the correlation of optical rotation and structure.

General reactions. Primary and secondary alcohols can be catalytically dehydrogenated with platinum and oxygen; 3-hydroxy-groups in steroids are oxidised,[19] but not those at position 6, 7, or 12. A study of the reduction of unsaturated steroids with deuterium has shown that in some cases deuterium may enter, not only at the positions on the double bond, but also at positions α to it. With Δ^5-steroids deuterium is introduced at the 5-, the 6-, and (to a smaller extent) the 7-position. This behaviour is ascribed [20] to the formation of a " platinonium " complex at $C_{(6)}$—$C_{(7)}$. Enol acetates with hypohalous acids yield α-halogenoketones.[20a]

Rings A and B.—The finding by the Schering group,[22, 23] that 1 : 4-dien-3-one analogues of the cortical hormones have antiarthritic properties, has focused attention on the grouping (4), hitherto of interest chiefly as a stage in the aromatisation of ring A. The 9α-fluoro-1 : 4-dien-3-ones promise to be of particular importance.[23, 24, 31, 32] The most commonly used indirect

[8a] J. Schreiber and A. Eschenmoser, *Helv. Chim. Acta*, 1955, **38**, 1529.
[9] R. N. Jones, F. Herling, and E. Katzenellenbogen, *J. Amer. Chem. Soc.*, 1955, **77**, 651; R. N. Jones, B. Nolin, and G. Roberts, *ibid.*, p. 6331.
[10] J. E. Page, *J.*, 1955, 2017.
[11] H. Rosenkrantz and P. Skogstrom, *J. Amer. Chem. Soc.*, 1955, **77**, 2237.
[12] D. H. W. Dickson, J. E. Page, and D. Rogers, *J.*, 1955, 443.
[13] M. Reggiani, B. Casu, and G. Garoti, *Gazzetta*, 1955, **85**, 1058.
[14] H. S. Wiggins and W. Klyne, *Chem. and Ind.*, 1955, 1448.
[15] R. C. Cookson and S. H. Dandegaonker, *J.*, 1955, 352.
[16] A. S. Meyer, *J. Org. Chem.*, 1955, **20**, 1240.
[17] C. Djerassi, E. W. Foltz, and A. E. Lippman, *J. Amer. Chem. Soc.*, 1955, **77**, 4354; E. W. Foltz, A. E. Lippman, and C. Djerassi, *ibid.*, p. 4359; A. E. Lippman, E. W. Foltz, and C. Djerassi, *ibid.*, p. 4364; C. Djerassi and R. Ehrlich, *ibid.*, 1956, **78**, 440.
[18] H. Rudolph, *J. Opt. Soc. Amer.*, 1955, **45**, 50.
[19] R. P. A. Sneeden and R. B. Turner, *J. Amer. Chem. Soc.*, 1955, **77**, 190.
[20] D. K. Fukushima and T. F. Gallagher, *ibid.*, p. 139.
[20a] B. J. Magerlein, D. A. Lyttle, and R. H. Levin, *J. Org. Chem.*, 1955, **20**, 1709.
[21] J. A. Hogg, F. H. Lincoln, A. H. Nathan, A. R. Hanze, W. P. Schneider, P. F. Beal, and J. Korman, *J. Amer. Chem. Soc.*, 1955, **77**, 4438.
[22] H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman, and M. M. Pechet, *Science*, 1955, **121**, 176; H. L. Herzog, C. C. Payne, M. A. Jevnik, D. Gould, E. L. Shapiro, E. P. Oliveto, and E. B. Hershberg, *J. Amer. Chem. Soc.*, 1955, **77**, 4781.
[23] A. Nobile, W. Charney, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. A. Jevnik, and E. B. Hershberg, *ibid.*, p. 4184.
[24] E. Vischer, C. Meystre, and A. Wettstein, *Helv. Chim. Acta*, 1955, **38**, 381, 1502.

Modified hormones.

Numbers indicate reference.

Modification	Cortisone, cortisol, or corticosterone	Progesterone, 11-deoxycorticosterone, or Reichstein's " S "	Testosterone and derivs.
Δ^1 *	21, 22,* 23,† 24 † 25, 25*a* †	23,† 24,† 26, 27	24, 26, 27
2-OH	28	28	—
2-Me	28*a*	—	—
5α-OH (instead of Δ^4)	29	29	—
Δ^8 and $\Delta^{(8)14}$	29*a*	—	—
9α-Halogen	—	30	30*a* ¶, 30*b* ‖
9α-F and Δ^1	21, 23,† 24,† 31, 32	—	—
Do., satd. at 4 : 5	31	—	—
9α-F and Δ^6	31, 32 ‡	—	—
$\Delta^{9(11)}$	—	—	33
12β-OH (instead of 11β)	34	—	—
14α-OH §	34*a* †	34*b* †	—
15α- and 15β-OH	—	34*c* †	—
Δ^{16}	35	—	—
16α-OH (instead of 17α)	36, 37	37	—
16β-OH	—	38	—
16-Oxo	—	—	38*a*
16α : 17α-Epoxy	35, 36	—	—
16-CH_2·	—	—	39
17α-Me	40	—	—
17β-Me (17α)	—	41	—
19-Nor	—	42	—
19-Oxo	—	43	—

* Prednisone (Metacortandracin; Meticorten) is 17α : 21-dihydroxypregna-1 : 4-diene-3 : 11 : 20-trione (1-dehydrocortisone). Prednisolone (Metacortandralone; Meticortelone) is the corresponding 11β-hydroxy-compound (1-dehydrocortisol).

† Microbiological method.

‡ Also 21-deoxy-compounds.

§ Also Δ^{14}-14 : 15-epoxy-, and 15β-halogeno-14α-hydroxy-compounds.

¶ Also carry 11β-hydroxy-groups.

‖ Also carry 11β-hydroxy- and 17α-methyl groups.

[25] S. Burstein and R. I. Dorfman, *J. Amer. Chem. Soc.*, 1955, **77**, 4668.

[25a] T. H. Stoudt, W. J. McAleer, J. M. Chemerda, M. A. Kozlowski, R. F. Hirschmann, V. Marlatt, and R. Miller, *Arch. Biochem. Biophys.*, 1955, **59**, 304.

[26] R. L. Clarke, K. Dobriner, A. Mooradian, and C. M. Martini, *J. Amer. Chem. Soc.*, 1955, **77**, 661.

[27] F. Sondheimer, M. Velasco, and G. Rosenkranz, *ibid.*, p. 5673.

[28] G. Rosenkranz, O. Mancera, and F. Sondheimer, *ibid.* p. 145.

[28a] J. A. Hogg, F. H. Lincoln, R. W. Jackson, and W. P. Schneider, *ibid.*, p. 6401.

[29] S. Bernstein and R. H. Lenhard, *ibid.*, p. 2233.

[29a] R. P. Graver, C. S. Snoddy, jun., and N. L. Wendler, *Chem. and Ind.*, 1956, 57.

[30] J. Fried, J. E. Herz, E. F. Sabo, A. Borman, F. M. Singer, and P. Numerof, *J. Amer. Chem. Soc.*, 1955, **77**, 1068.

[30a] R. H. Lenhard and S. Bernstein, *ibid.*, p. 6665.

[30b] M. E. Herr, J. A. Hogg, and R. H. Levin, *ibid.*, 1956, **78**, 500.

[31] R. F. Hirschmann, R. Miller, R. E. Beyler, L. H. Sarett, and M. Tishler, *ibid.*, p. 3166.

[32] J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman, and F. M. Singer, *ibid.*, p. 4181.

[33] F. W. Heyl and M. E. Herr, *ibid.*, p. 488.

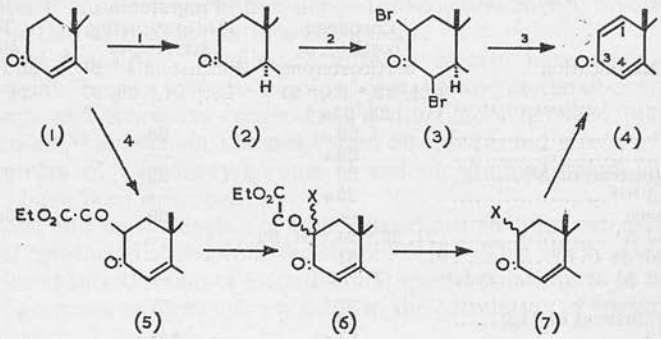
[34] W. J. Adams, D. N. Kirk, D. K. Patel, V. Petrow, and I. A. Stuart-Webb, *J.*, 1955, 870.

[34a] E. J. Agnello, B. M. Bloom, and G. D. Laubach, *J. Amer. Chem. Soc.*, 1955, **77**, 4684.

[34b] B. M. Bloom, E. J. Agnello, and G. D. Laubach, *Experientia*, 1956, **12**, 27.

[34c] S. Bernstein, L. I. Feldman, W. S. Allen, R. H. Blank, and C. E. Linden, *Chem. and Ind.*, 1956, 111.

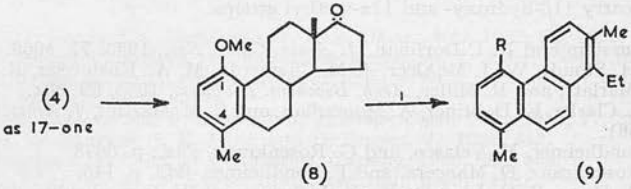
method for the introduction of the 1 : 2-double bond is by dibromination of a saturated 3-oxo-steroid and treatment with collidine (1 ⟶ 4).[22, 27, 31, 32]



Reagents: 1, H_2. 2, Br_2. 3, Collidine. 4, Ethyl oxalate.

Another method [21] involves condensation of a 4-en-3-one (1) with ethyl oxalate, followed by bromination, hydrolysis, and debromination [(5) ⟶ (6; X = Br) ⟶ (7; X = Br) ⟶ (4)]. The 1 : 2-double bond may be introduced directly by micro-organisms [23, 24] and (in poor yield) by treatment of 4-en-3-ones with lead tetra-acetate.[25, 26] The last-mentioned reaction yields chiefly 2-acetoxy-4-en-3-ones, which may also be obtained by rearrangement of the 6-bromo-derivatives with potassium acetate in acetic acid.[26]

The Upjohn group [28a] have recently found that the introduction of a 2-methyl group into cortisol or 9α-fluorocortisol gives compounds with very high biological activity (in both the glycogen-deposition and the salt-retention test). The 2-methyl group is introduced *via* the ethoxalyl compound [(5) ⟶ (6; X = Me) ⟶ (7; X = Me)].



Reagent: 1, Pd–C at 300°.

Dehydrogenation (palladium–charcoal at 300°) of the phenolic steroid (8), obtained by aromatisation of a 1 : 4-dien-3-one (4) in the dienone–phenol rearrangement, gives among other products two phenanthrene derivatives

[35] W. F. McGuckin and H. L. Mason, *J. Amer. Chem. Soc.*, 1955, **77**, 1822.
[36] P. L. Julian, W. Cole, E. W. Meyer, and B. M. Regan, *ibid.*, p. 4601.
[37] B. Ellis, F. Hartley, V. Petrow, and D. Wedlake, *J.*, 1955, 4383.
[38] S. Bernstein, M. Heller, and S. M. Stolar, *J. Amer. Chem. Soc.*, 1955, **77**, 5327.
[38a] W. J. Adams, D. K. Patel, V. Petrow, and I. A. Stuart-Webb, *J.*, 1956, 296.
[39] F. Neuman, O. Mancera, G. Rosenkranz, and F. Sondheimer, *J. Amer. Chem. Soc.*, 1955, **77**, 5676.
[40] C. R. Engel, *ibid.*, p. 1064.
[41] C. R. Engel and G. Just, *Canad. J. Chem.*, 1955, **33**, 1515.
[42] A. Sandoval, G. H. Thomas, C. Djerassi, G. Rosenkranz, and F. Sondheimer, *J. Amer. Chem. Soc.*, 1955, **77**, 148.
[43] G. W. Barber and M. Ehrenstein, *J. Org. Chem.*, 1955, **20**, 1253.

(9; R = H or OMe) whose structures show that the phenol (8) is a 1-methoxy-4-methyl derivative (*not* a 4-methoxy-1-methyl compound).[44]

Studies of 2 : 3-diols and related compounds have been made in the 5α-series [45, 46] and in the 5β-series [47]—in each case in connexion with the stereochemistry of sapogenins. Gitogenin is 2α : 3β-dihydroxy-5α : 22*a* : 25*D*-spirostan (10); [45, 46] samogenin is 2β : 3β-dihydroxy-5β : 22*a* : 25*D*-spirostan (11).[47] The Prévost reagent (silver acetate and iodine in wet acetic acid) transforms a Δ^2-5α-compound into the 2β : 3β-diol [45] (osmium tetroxide gives the 2α : 3α-diol). Both the 2α : 3α- and the 2α : 3β-di(methanesulphonate) with sodium iodide and acetone at 100° yield a 2-ene.[46]

Two convenient partial syntheses of androsterone (12) have been described. One involves the sequence of 3β-toluene-*p*-sulphonate ⟶ 2-ene ⟶ 2α : 3α-epoxide ⟶ 3α-hydroxy-compound; [48] the other consists of selective hydrogenation of 5α-androstane-3 : 17-dione with deactivated Raney nickel.[49]

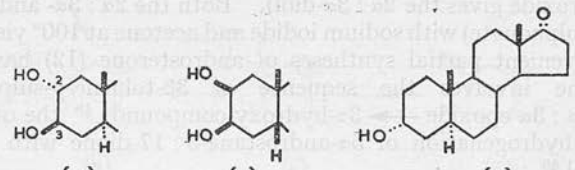
Saturated 3-ketones form dimethyl ketals with selenium dioxide in methanol,[50] whilst 11-, 17-, 20-, and Δ^4-3-keto-groups do not react. This reaction has been used in a synthesis of cortisol.[51] 3-Hydroxy-groups can be oxidised by *tert.*-butyl hypochlorite.[52]

Shoppee and his colleagues have described [53] kinetic studies of a number of replacement reactions at $C_{(3)}$. They have also discussed the conditions in which 3β-substituted Δ^5-steroids undergo S_N2 reactions to give 3α-Δ^5 compounds; [54] powerful nucleophilic reagents in media of low dielectric constant are necessary (six examples are quoted). In other conditions replacement reactions yield 3β-Δ^5- or 6β-3 : 5-*cyclo*-compounds. Related work on rearrangements of 3α-Δ^5-derivatives has been reported by Becker and Wallis.[55]

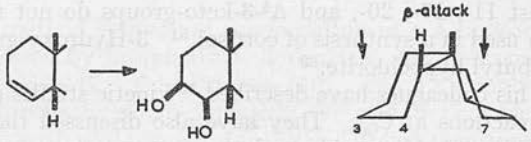
The stereochemistry of 3-amino-steroids is now well established; cholesterylamine (3β-aminocholest-5-ene) has been obtained [56] by degradation of the Δ^5-3β-carboxylic acid discussed last year. Work on reaction mechanisms provides further evidence regarding configurations.[54, 57] Δ^4-3-Amino-steroids,[58] 2- and 6-amino-steroids,[59] and a number of nitrogen analogues of the androgens [60] have been described.

[44] A. S. Dreiding and A. Voltman, *J. Amer. Chem. Soc.*, 1954, **76**, 537; A. S. Dreiding and A. J. Tomasewski, *ibid.*, p. 540.
[45] C. Djerassi, L. B. High, T. T. Grossnickle, and R. Ehrlich, *Chem. and Ind.*, 1955, 474.
[46] N. L. Wendler and H. L. Slates, *ibid.*, 1954, 167.
[47] C. Djerassi and J. Fishman, *J. Amer. Chem. Soc.*, 1955, **77**, 4291.
[48] J. Iriarte, G. Rosenkranz, and F. Sondheimer, *J. Org. Chem.*, 1955, **20**, 542.
[49] C. Djerassi, A. J. Manson, and M. Gorman, *J. Amer. Chem. Soc.*, 1955, **77**, 4925.
[50] E. P. Oliveto, C. Gerold, and E. B. Hershberg, *ibid.*, 1954, **76**, 6113.
[51] E. P. Oliveto, H. Q. Smith, C. Gerold, L. Weber, R. Rausser, and E. B. Hershberg, *ibid.*, 1955, **77**, 2224.
[52] G. S. Fonken, J. L. Thompson, and R. H. Levin, *ibid.*, p. 172.
[53] R. H. Davies, S. Meecham, and C. W. Shoppee, *J.*, 1955, 679; C. W. Shoppee and D. F. Williams, *ibid.*, p. 686; C. W. Shoppee and D. T. Westcott, *ibid.*, p. 1891.
[54] J. H. Pierce, H. C. Richards, C. W. Shoppee, R. J. Stephenson, and G. H. R. Summers, *ibid.*, p. 694.
[55] E. J. Becker and E. S. Wallis, *J. Org. Chem.*, 1955, **20**, 353.
[56] J. H. Pierce, C. W. Shoppee, and G. H. R. Summers, *J.*, 1955, 690.
[57] L. Labler and F. Šorm, *Coll. Czech. Chem. Comm.*, 1955, **20**, 188; R. D. Haworth, L. H. C. Lunts, and J. McKenna, *J.*, 1955, 986.
[58] R. A. B. Bannard and A. F. McKay, *Canad. J. Chem.*, 1955, **33**, 1166.
[59] C. W. Bird and R. C. Cookson, *Chem. and Ind.*, 1955, 1479.
[60] J. Joska and F. Šorm, *Chem. Listy*, 1955, **49**, 1687.

The pyrolysis of 3α-hydroxy-5β-cholanic acid (lithocholic acid) yields chiefly 5β-chol-3-enic acid (13). Treatment of this with osmium tetroxide gives as the main product the 3β : 4β-diol (14) (with a little of the 3α : 4α-diol).[61] The " front attack " on ring A of a 5β-compound contrasts with a " rear attack " (at nearly all positions) of a 5α-compound.[47, 61] Formula (15) shows clearly that the β-face of ring A is the more open to attack. This is true also for ring B : 7-oxo-5β-cholanic acid with sodium borohydride gives almost entirely the 7α-hydroxy-acid.[62]



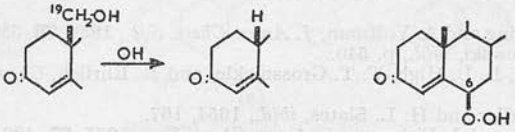
(10) (11) (12)



(13) (14) (15)

A thorough study of the 4 : 5- and the 5 : 6-diol, and of the allylic alcohols of Δ^4-6-ol and Δ^5-4-ol type,[63, 55] has cleared up the nature of the rearrangement of cholest-5-en-3α-yl toluene-*p*-sulphonate with potassium acetate in methanol.

19-Hydroxy-4-ene-3-ones (16), on treatment with alkali, lose formaldehyde, to give 19-nor-compounds (17) [64, 43] (cf. also ref. 150). This reaction resembles, vinylogously, eliminations from β-ketols in the triterpene series.[65]



(16) (17) (18)

Treatment of cholest-5-en-3-one with molecular oxygen in hexane at 25° yields a carcinogen [66] which is apparently the hydroperoxide (18).

[61] K. Yamasaki, V. Rosnati, M. Fieser, and L. F. Fieser, *J. Amer. Chem. Soc.*, 1955, **77**, 3308.

[62] E. H. Mosbach, W. Meyer, and F. E. Kendall, *ibid.*, 1954, **76**, 5799. A further example of β-attack (not strictly analogous to the above) is the formation of a 5β : 6β-diol by the action of osmium tetroxide on a Δ^5-3-ethylene ketal (S. Bernstein, W. S. Allen, C. E. Linden, and J. Clemente, *ibid.*, 1955, **77**, 6612).

[63] D. N. Jones, J. R. Lewis, C. W. Shoppee, and G. H. R. Summers, *J.*, 1955, 2870.

[64] A. S. Meyer, *Experientia*, 1955, **11**, 99.

[65] D. H. R. Barton and P. de Mayo, *J.*, 1954, 887.

[66] L. F. Fieser, T. W. Greene, F. Bischoff, G. Lopez, and J. J. Rupp, *J. Amer. Chem. Soc.*, 1955, **77**, 3928.

Three groups of workers [67] have independently described an ingenious preparation of Δ^5-3β-hydroxy-compounds from 3α : 6α-diols of the 5β-series (such as hyodeoxycholic acid).

Other work on A-ring reactions includes further studies on ring contraction by the Favorskii reaction,[68] studies on polybromocholestan-3-ones,[69] 4-halogeno-Δ^4-3-ketones,[70] 6β-iodo-Δ^4-3-ketones,[71] oestrene-3 : 17β-diols [with 5(10)-, 4-, or 5-double bond],[72] and several papers on di-steroids.[73] Further work has been carried out on bromination of 6-ketones [74] and 7-ketones (both 5α- and 5β-).[75, 76] Dehydrobromination of 7-bromocholest-5-en-3β-yl benzoate yields the 6 : 8(14)-dien-3β-ol and the 4 : 6 : 8(14)-triene.[77]

4 : 6-Dien-3-ones can be hydrogenated selectively to 4-en-3-ones or to 5β-3-ketones [78] by various palladium catalysts in alkaline conditions. A re-investigation of the so-called "*i*-cholestane-6 : 7-diacids" [79] shows that only one of them is a 3α : 5-*cyclo*-6 : 7-*seco*cholestane-6 : 7-dioic acid (19). Extensive studies have been carried out on epidioxides from lumisterol and related compounds.[80] "*pseudo*Cholesterol" is cholest-4-en-7β-ol.[81]

Several reports of the introduction of tertiary hydroxyl groups at $C_{(8)}$ or $C_{(9)}$ (probably 8β) by microbiological reactions have appeared.[4, 4a, 82]

Several studies have appeared on the aromatisation of ring B, with certain or probable displacement of ring A. 5 : 8-Dien-7-ones (20) on treatment with acidic reagents are rearranged to phenols.[80, 83] In one case the phenol was subsequently dehydrogenated to a mixture of anthracene derivatives, which proves the structure (21) [83] (cf. the dienone–phenol rearrangement in ring A). In the other case (ergostane and lumistane series) [80] the alternative course with migration of the angular methyl group to give a 6-methyl-19-nor-steroid cannot be excluded.

[67] L. Vargha and M. Rados, *Chem. and Ind.*, 1955, 896; P. Ziegler and K. R. Bharucha, *ibid.*, p. 1351; S. Bergstrom and K. Paabo, *Acta Chem. Scand.*, 1955, **9**, 699; L. Vargha, M. Rados, and M. Kraut, *Acta Chim. Acad. Sci. Hung.*, 1955, **8**, 303.

[68] B. B. Smith and H. R. Nace, *J. Amer. Chem. Soc.*, 1954, **76**, 6119; D. E. Evans, A. C. de Paulet, C. W. Shoppee, and F. Winternitz, *Chem. and Ind.*, 1955, 355.

[69] M. Fieser, M. A. Romero, and L. F. Fieser, *J. Amer. Chem. Soc.*, 1955, **77**, 3305.

[70] J. I. Shaw and R. Stevenson, *J.*, 1955, 3459.

[71] C. Djerassi, J. Grossman, and G. H. Thomas, *J. Amer. Chem. Soc.*, 1955, **77**, 3826.

[72] J. A. Hartman, *ibid.*, p. 5151.

[73] E. J. Corey and R. L. Young, *ibid.*, p. 1672; J. Chopin, *Compt. rend.*, 1955, **240**, 201; *Ann. Chim.* (*France*), 1954, **9**, 605; Y. Raoul, N. Le Boulch, C. Baron, J. Chopin, and A. Guerillot-Vinet, *Bull. Soc. Chim. biol.*, 1954, **36**, 1265.

[74] D. R. James and C. W. Shoppee, *J.*, 1954, 4224.

[75] K. Tsuda and R. Hayatsu, *J. Amer. Chem. Soc.*, 1955, **77**, 665.

[76] K. Takeda, K. Igarashi, and T. Komeno, *Pharm. Bull.* (*Japan*), 1954, **2**, 348; K. Takeda, T. Komeno, and K. Igarashi, *ibid.*, p. 352.

[77] F. Hunziker, F. X. Müllner, K. G. Reuteler, and H. Schaltegger, *Helv. Chim. Acta*, 1955, **38**, 1316.

[78] G. Slomp, jun., Y. F. Shealy, J. L. Johnson, R. A. Donia, B. A. Johnson, R. P. Holysz, R. L. Pederson, A. O. Jensen, and A. C. Ott, *J. Amer. Chem. Soc.*, 1955, **77**, 1216; D. A. Shepherd, R. A. Donia, J. A. Campbell, B. A. Johnson, R. P. Holysz, G. Slomp, jun., J. E. Stafford, R. L. Pederson, and A. C. Ott, *ibid.*, p. 1212.

[79] S. Gates and E. S. Wallis, *J. Org. Chem.*, 1955, **20**, 610.

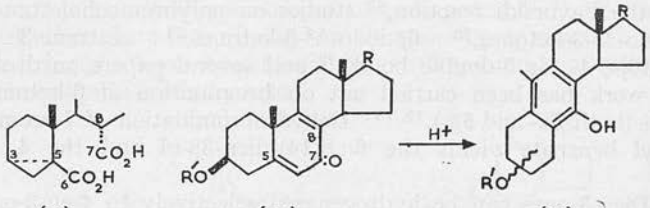
[80] P. Bladon, *J.*, 1955, 2176.

[81] Q. R. Petersen and C. T. Chen, *J. Amer. Chem. Soc.*, 1955, **77**, 2557; R. J. W. Cremlyn, R. W. Rees, and C. W. Shoppee, *J.*, 1954, 3790.

[82] D. Stone, M. Hayano, R. I. Dorfman, O. Hechter, C. R. Robinson, and C. Djerassi, *J. Amer. Chem. Soc.*, 1955, **77**, 3926.

[83] K. Tsuda, K. Arima, and R. Hayatsu, *ibid.*, 1954, **76**, 2933.

Dehydroergosteryl acetate, a 5 : 7 : 9(11)-triene, is rearranged by hydrogen chloride in chloroform to a benzenoid compound; [84] this on dehydrogenation gives a product which shows absorption of the anthracene type, and on



(19) (20) (21)

(R'O at 3α or 2β).

oxidation with nitric acid gives 3-methylbenzene-1 : 2 : 4 : 5-tetracarboxylic acid. The benzenoid product is therefore of type (21)*, formed by fission of the 1 : 10-bond. (An alternative very similar structure formed by fission of the 9 : 11-bond cannot be entirely excluded.) Characterisation of "anthra"- and "neo"-steroids by infrared and ultraviolet spectra has been discussed.[85] 5 : 7-, 6 : 8-, and 5 : 8-Dienes, on irradiation in presence of mercuric acetate and toluene-*p*-sulphonic acid in nitrogen, yield benzenoid compounds,[86] presumably of type (21), which can be dehydrogenated to anthracenes.

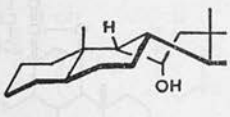
Ring C.—Several papers on the synthesis of cortisone and cortisol are concerned with improvements in yield and changes in the order in which functional groups are introduced. They deal with, *inter alia*, the use of 11β-formates [87] and the transformation of 11α- into 11β-hydroxy-compounds *via* the 11-ketones [88] (see also refs. 51, 130, 131).

The "direct" synthesis of cortisone and cortisol from 11-oxoprogesterone by the Upjohn group [89] is of particular interest in that the Δ^4-3-ketone group is maintained (protected by enamine or ketal formation) throughout the synthesis. The side-chain reactions of this process are considered on p. 220. Contrary to previous findings, 11-oxo-groups can form ethylene ketals although the reaction is very slow.[90]

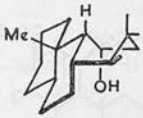
11-Oxo-5α-steroids, on bromination, yield 9α-bromo-compounds, which on treatment with lithium aluminium hydride give 11β-hydroxy-compounds and 9β : 11β-epoxides.[91] Henbest, Jones, and their colleagues have studied reactions at $C_{(11)}$ in 11-oxo-9β-steroids.[92] Apparently the β-face is more

[84] W. R. Nes and E. Mosettig, *J. Amer. Chem. Soc.*, 1955, **77**, pp. 3182, 3186; W. R. Nes, *ibid.*, 1956, **78**, 193; W. R. Nes, R. B. Kostic, and E. Mosettig, *ibid.*, p. 436.
[85] I. Scheer, W. R. Nes, and P. B. Smeltzer, *ibid.*, 1955, **77**, 3300.
[86] K. Tsuda and R. Hayatsu, *ibid.*, p. 3089.
[87] E. P. Oliveto, C. Gerold, R. Rausser, and E. B. Hershberg, *ibid.*, p. 3564.
[88] W. S. Allen, S. Bernstein, and R. Littell, *ibid.*, 1954, **76**, 6116; S. Bernstein and R. H. Lenhard, *ibid.*, 1955, **77**, 2331.
[89] J. A. Hogg, P. F. Beal, A. H. Nathan, F. H. Lincoln, W. P. Schneider, B. J. Magerlein, A. R. Hanze, and R. W. Jackson, *ibid.*, p. 4436.
[90] B. J. Magerlein and R. H. Levin, *ibid.*, p. 1904.
[91] H. B. Henbest, E. R. H. Jones, A. A. Wagland, and T. I. Wrigley, *J.*, 1955, 247[illegible]
[92] A. Crawshaw, H. B. Henbest, E. R. H. Jones, and A. A. Wagland, *J.*, 1955, 342[illegible]
* Without R'O and OH, and with one additional double bond conjugated with the aromatic nucleus.

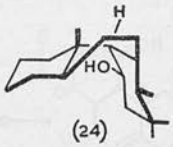
accessible (possible conformations are 22, 23, 24); on reduction, 11α-hydroxy-9β-compounds are formed. The saturated 9β-compounds (without substituents in ring B or C) are accessible by catalytic hydrogenation of 9β-11-enes. An 11-oxo-9β-diene (probably $\Delta^{8(14):15}$) has also been described.[93]



(22)
(C-boat).



(23)
(C-boat).

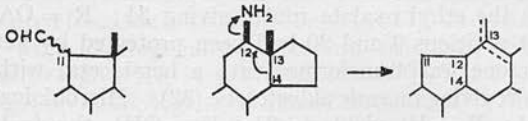


(24)
(B-boat).

The Basle school have carried out a thorough study of 11 : 12-ketols,[94] which are of interest in connexion with various cardiac aglycones. 11β-Acetoxy-12-ketones and 12α-acetoxy-11-ketones (acetoxy-groups axial), on treatment with zinc and boiling glacial acetic acid, give the 12- and 11-ketones respectively in good yield. The corresponding equatorial ketol acetates give poor yields; a mechanism is suggested.[95] An improved method for deacetoxylation of a 12β-acetoxy-11-oxo-compound in the spirostan series is described by the Glaxo group,[96] *viz.*, treatment with calcium and liquid ammonia in tetrahydrofuran or benzene. (The free ketol in the same conditions yields the 11α : 12β-diol.)

The configurations of 11 : 12-disubstituted sapogenins, and the transformation of the diequatorial 11α : 12β-derivatives by ring contraction to 11-formyl-C-nor-compounds (25), have been further discussed.[97]

A further study of the C-nor-D-homo ("jervine") rearrangement has been made, using the Demjanow reaction of a 12β-aminospirostan (26) with nitrous acid.[98]



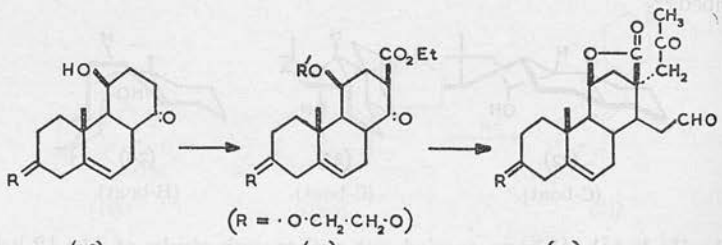
(25) (26) (27)

An ingenious modified Dische reaction based on a formal similarity between the $C_{(11)}$–$C_{(17)}$ grouping in cortisone, cortisol, aldosterone, etc., and lævulic acid provides a potentially valuable specific reaction for these hormones and some related compounds.[99]

Aldosterone.—Wettstein and his colleagues (Ciba) have described[100] the total synthesis of racemic aldosterone (32) from Sarett's tricyclic inter-

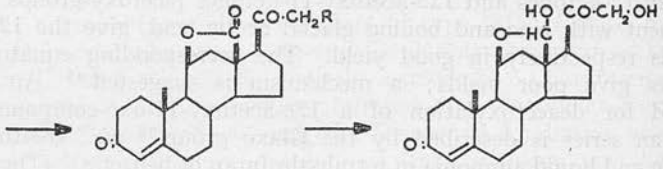
[93] J. Grigor, G. T. Newbold, and F. S. Spring, *J.*, 1955, 1170.
[94] G. Baumgartner and C. Tamm, *Helv. Chim. Acta*, 1955, **38**, 441.
[95] R. S. Rosenfeld and T. F. Gallagher, *J. Amer. Chem. Soc.*, 1955, **77**, 4367.
[96] J. H. Chapman, J. Elks, and L. J. Wyman, *Chem. and Ind.*, 1955, 603.
[97] N. L. Wendler, R. F. Hirschmann, H. L. Slates, and R. W. Walker, *J. Amer. Chem. Soc.*, 1955, **77**, 1632.
[98] R. Anliker, O. Rohr, and H. Heusser, *Helv. Chim. Acta*, 1955, **38**, 1171.
[99] I. Clark, *Nature*, 1955, **175**, 123.
[100] J. Schmidlin, G. Anner, J. R. Billeter, and A. Wettstein, *Experientia*, 1955, **11**, 365.

mediate [101] (28) in about 20 stages, by methods generally resembling those used by the Merck group for cortisone.[102] The essential difference lay in the introduction of the oxygen function at $C_{(18)}$ as ethoxycarbonyl, which subsequently formed an 18→11-lactone.



(R = ·O·CH$_2$·CH$_2$·O)

(28) (29) (30)



(31) (32)

(29) ⟶ (30), 11 stages. (30) ⟶ (31), 3 stages. (31; R = H) ⟶ (31; R = OAc), 3 stages. (31) ⟶ (32), 3 stages.

Ring D was then built on by the 16-en-20-one procedure (cf. p. 229), to give (racemic) (31; R = H) corresponding to the natural product obtained by degradation of aldosterone.[103] The 21-hydroxyl function was introduced by the ethyl oxalate route (giving 31; R = OAc); after the keto-groups at positions 3 and 20 had been protected by ketal formation, the 18→11-lactone was transformed into a hemiacetal with lithium aluminium hydride, giving racemic aldosterone (32). Microbiological hydroxylation of (±31; R = H) yields (+31; R = OH), the (−)-isomer being unattacked.[102a] Since (+31; R = OH) has been converted into (+)-aldosterone,[103] this completes the synthesis of the latter in its natural form.

New degradations and partial syntheses in the aldosterone series involving the 11-, 18-, and 20-oxygen functions have been described.[103, 104] Heusser and his colleagues have prepared intermediates which it is hoped will serve for the introduction of an 18-oxygen function into existing steroids.[105, 106]

[101] G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Amer. Chem. Soc.*, 1953, **75**, 422.

[102] G. E. Arth, G. I. Poos, R. M. Lukes, F. M. Robinson, W. F. Johns, M. Feurer, and L. H. Sarett, *ibid.*, 1954, **76**, 1715; G. I. Poos, R. M. Lukes, G. E. Arth, and L. H. Sarett, *ibid.*, p. 5031.

[102a] E. Vischer, J. Schmidlin, and A. Wettstein, *Experienta*, 1956, **12**, 50.

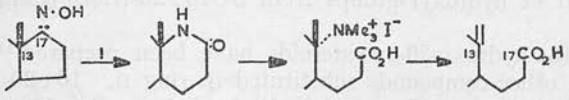
[103] J. von Euw, R. Neher, and T. Reichstein, *Helv. Chim. Acta*, 1955, **38**, 1423.

[104] E. A. Ham, R. E. Harman, N. G. Brink, and L. H. Sarett, *J. Amer. Chem. Soc.*, 1955, **77**, 1637.

[105] H. Heusser, J. Wohlfahrt, M. Müller, and R. Anliker, *Helv. Chim. Acta*, 1955, **38**, 1399.

[106] R. Anliker, M. Müller, J. Wohlfahrt, and H. Heusser, *ibid.*, p. 1404.

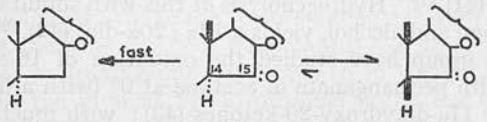
The oxime of a 17-oxo-steroid (33), by Beckmann rearrangement, yields the lactam (34); Hofmann degradation of the related quaternary ammonium salt (35) yields a 13(18)-methylene compound (36). Similar reactions in the D-homo-series are also described. The introduction of 18-hydroxy-groups into 11-deoxycorticosterone by ox adrenal homogenates has been reported.[107]



(33) (34) (35) (36)

Reagent: I, $AcHN{\cdot}C_6H_4{\cdot}SO_2Cl$.

Ring D and the Pregnane Side-chain.—Studies on the stereochemistry of digitogenin (a 2 : 3 : 15-trihydroxy-5α : 22*a* : 25*D*-spirostan) by the schools of Djerassi and Fieser have raised some interesting points. Proof that



(39) (37) (38)

gitogenin is a 2α : 3β-diol [47,48] shows that digitogenin also must have the 2α : 3β-diol grouping.[108] Alkaline isomerisation of digitogenone (37) gives a 14-*epi*-compound (38). Wolff–Kishner reduction of either of these 15-ketones yields the 14α-compound gitogenin (39); this is explained [109,110] as follows: the less stable 14α-15-ketone (37) is reduced so much faster than the 14β-15-ketone (38) that, although the latter predominates at equilibrium in the absence of reducing agent, the 14α-deoxo-compound (39) is the only product isolated from the reduction. 5α-(14α : 17β)-Pregnane-3 : 15 : 20-trione (formed from digitogenin by standard reactions) mutarotates in alkaline methanol to give the 14β : 17α-triketone.[109] The configuration of the 15-hydroxy-group in digitogenin is almost certainly β.[108,109,110]

20-Oxo-14 : 16-dienes on reduction with metals and propanol yield 20-(α and β)-hydroxy-14-enes with normal configuration at $C_{(17)}$.[111]

Thorough studies by Šorm and his school have clarified the stereochemistry of $C_{(16)}$ in the 5α-androstane series by reference to the known stereochemistry at $C_{(17)}$,[112] and similar work has been done in the androstane and œstratriene series.[113] Huffmann and his colleagues have provided an improved route to 16-oxo-steroids which involves reduction of a 16-oxo-

[107] F. W. Kahnt, R. Neher, and A. Wettstein, *Helv. Chim. Acta.*, 1955, **38**, 1237. 19-Hydroxy-groups can also be introduced (see ref. 4*b*). Subsequently the same workers showed that aldosterone can be so formed (*Experientia*, 1955, **11**, 446).

[108] D. L. Klass, M. Fieser, and L. F. Fieser, *J. Amer. Chem. Soc.*, 1955, **77**, 3829.

[109] C. Djerassi, L. B. High, J. Fried, and E. F. Sabo, *ibid.*, p. 3673.

[110] Cf. ref. 4*b*.

[111] H. Heusser, M. Roth, O. Rohr, and R. Anliker, *Helv. Chim. Acta*, 1955, **38**, 1178.

[112] J. Fajkos, *Coll. Czech. Chem. Comm.*, 1955, **20**, 312, 1478; J. Fajkos and F. Šorm, *ibid.*, p. 1464. Two compounds previously isolated from mare's urine are identical with 5α-androstane-3β : 16α(and 16β)-diols (R. V. Brooks and W. Klyne, *Biochem. J.*, 1956, **62**, 21P).

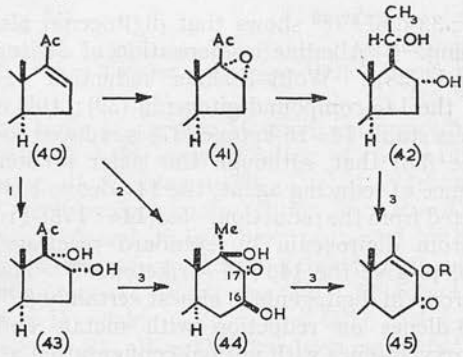
[113] M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, 1955, **213**, 343; **215**, 627, 633.

steroid 17β-toluene-*p*-sulphonate with sodium borohydride to the 16β-hydroxy-analogue; the latter on treatment with alcoholic alkali yields the 16-oxo-compound (without a 17-substituent).[114] 16-*epi*Oestriol (a 3 : 16β : 17β-triol) has been isolated from pregnancy urine.[115] 5α- and 5β-Cholestane and their 16β-hydroxy-derivatives have been obtained by stepwise removal of hydroxyl groups from 3 : 16 : 26-triols (tetrahydrosapogenins).[116]

Several 16α-hydroxy-20-oxo-steroids have been prepared[117] and correlated with other compounds substituted in ring D. 16 : 20-Dioxo- and 16β-hydroxy-20-oxo-steroids can be obtained from the 16α-hydroxy-20-oxo-compounds by treatment of the 20-ethylene ketals with chromium trioxide–pyridine, followed by lithium aluminium hydride.[118] Other work on 16 : 20-disubstituted compounds is mentioned in the section on total synthesis (see refs. 189, 190).

Whilst a 16-en-20-one (40) reacts with alkaline methanol in the absence of air to give a 16β-methoxy-compound, in the presence of air it gives the 16 : 17-epoxide (41).[119] Hydrogenolysis of this with simultaneous reduction at $C_{(20)}$, by sodium and alcohol, yields a 16α : 20α-diol (42).[120]

The B.D.H. group have studied the oxidation of 16-en-20-ones (40); on treatment with permanganate in acetone at 0° (with a little acetic acid) these yield 16α : 17α-dihydroxy-20-ketones (43); with much acetic acid the corresponding Δ^{14}-compounds are obtained.[121]

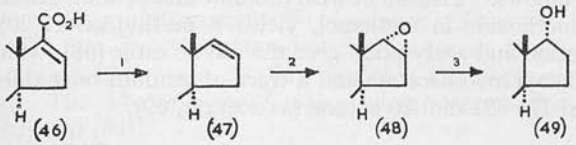


Reagents: 1, $KMnO_4$. 2, OsO_4. 3, OH^-.

The 16-en-20-ones with osmium tetroxide in pyridine yield 16 : 17-dihydroxy-17-oxo-D-homo-compounds (44 and isomers); these are also produced from 16α(and β) : 17α-dihydroxy-20-ketones on treatment with alkali (or alkaline alumina).[121, 122]

[114] M. N. Huffman, M. H. Lott, and A. Tillotson, *J. Biol. Chem.*, 1955, **217**, 103, 107.
[115] G. F. Marrian and W. S. Bauld, *Biochem. J.*, 1955, **59**, 136.
[116] I. Scheer and E. Mosettig, *J. Amer. Chem. Soc.*, 1955, **77**, 1820.
[117] H. Hirschmann, F. B. Hirschmann, and J. W. Corcoran, *J. Org. Chem.*, 1955, **20**, 572.
[118] S. Bernstein, M. Heller, and S. N. Stolar, *J. Amer. Chem. Soc.*, 1955, **77**, 5327.
[119] G. P. Mueller and L. L. Norton, *ibid.*, p. 143.
[120] B. Camerino and C. G. Alberti, *Gazzetta*, 1955, **85**, 56.
[121] G. Cooley, B. Ellis, F. Hartley, and V. Petrow, *J.*, 1955, 4373, 4377.
[122] Cf. H. H. Inhoffen, F. Blomeyer, and K. Bruckner, *Chem. Ber.*, 1954, **87**, 583; K. Heusler and A. Wettstein, *ibid.*, p. 1301.

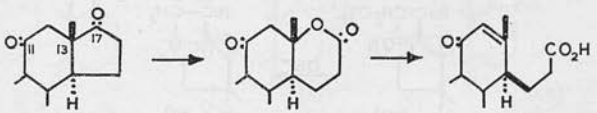
Work from the Syntex laboratories has provided the first easy route to Δ^{16}-steroids of the $C_{(19)}$ series (47) and thence *via* 16α : 17α-epoxides to 17α-hydroxy-compounds (49). The essential step is the decarboxylation of the Δ^{16}-17-carboxylic acid (46).[123] Similar reaction sequences lead from $\Delta^{17(20)}$-21-oic acids and $\Delta^{17(20)}$-enes to the 17α-hydroxy-17β-methyl (and ethyl) compounds.



Reagents : 1, $CuCr_2O_4$–quinoline. 2, BzO_2H. 3, $LiAlH_4$.

The 17-hydroxy-group may be removed from side-chains of dihydroxyacetone type by dehydration of the 20-ethylene ketal 21-acetate with thionyl chloride in pyridine at −5° to the corresponding Δ^{16}-compound (phosphorus oxychloride in pyridine at room temperature has no action).[124] Replacement of the 17-hydroxy-group by hydrogen in similar structures can be carried out *via* the glyoxal 21 : 21-dibenzyl acetal and hydrogenolysis of this.[125]

The structure of the lactones obtained by oxidation of 17-oxo-steroids (50) with perbenzoic acid has been proved; the compound (51), obtained



from an 11 : 17-dioxo-steroid yields the αβ-unsaturated ketone (52) on treatment with sodium methoxide in methanol.[126]

Reduction of Δ^{16}-20-ketones by lithium aluminium hydride gives largely the Δ^{16}-20α-hydroxy-compounds.[127] Most other types of 20-ketones give 20β-ols (but see ref. 128). With 20-ketones of several different types sodium borohydride yields 20β-ols ; [129] the 20(or 17)-keto-group is reduced in preference to the Δ^4-3-ketone group.

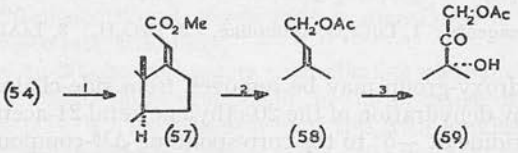
Ruschig [130] has described the condensation of 20-ketones with ethyl oxalate, followed by iodination and acid hydrolysis, to give 21-iodo-ketones

[123] F. Sondheimer, O. Mancera, M. Urquiza, and G. Rosenkranz, *J. Amer. Chem. Soc.*, 1955, **77**, 4145; E. Batres, G. Rosenkranz, and F. Sondheimer, *ibid.*, p. 4155.
[124] W. S. Allen and S. Bernstein, *J. Amer. Chem. Soc.*, 1955, **77**, 1028; S. A. Szpilfoge and V. Gerris, *Rec. Trav. chim.*, 1955, **74**, 1462.
[125] O. Mancera, G. Rosenkranz, and F. Sondheimer, *J. Amer. Chem. Soc.*, 1955, **77** 5669; cf. ref. 1, pp. 234—235.
[126] N. L. Wendler, D. Taub, and H. L. Slates, *J. Amer. Chem. Soc.*, 1955, **77**, 3559.
[127] E. L. Shapiro, D. Gould, and E. B. Hershberg, *ibid.*, p. 2912.
[128] G. I. Poos, *ibid.*, p. 4932.
[129] J. K. Norymberski and G. F. Woods, *J.*, 1955, 3426; B. Camerino and C. G. Alberti, *Gazzetta*, 1955, **85**, 51.
[130] H. Ruschig, *Angew. Chem.*, 1948, **60**, 247; *Chem. Ber.*, 1955, **88**, 878.

(53—56) and thence 21-acetoxy-ketones. This method has been used by other workers [131] and has also been applied at position 2 (see p. 214).

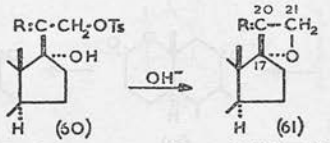
$R{\cdot}CO{\cdot}CH_3$	$R{\cdot}CO{\cdot}CH_2{\cdot}CO{\cdot}CO_2Et$	$R{\cdot}CO{\cdot}CHI{\cdot}CO{\cdot}CO_2Et$	$R{\cdot}CO{\cdot}CH_2I$
(53)	(54)	(55)	(56)

The Upjohn " direct " synthesis of cortisone [89] uses the ethoxalyl derivative (54) as follows. Treatment with bromine and sodium acetate, followed by sodium methoxide in methanol, yields a methyl *cis*-17(20)-en-21-oate (57). Reduction and acetylation give the allylic ester (58), which on treatment with phenyl iodosoacetate and a trace of osmium tetroxide is oxidised to the desired 17α : 21-diol-20-ketone (as acetate, 59).



Reagents: 1 (*a*) Br, (*b*) NaOMe. 2 (*a*) $LiAlH_4$, (*b*) Ac_2O. 3, $PhI(OAc)_2$–OsO_4.

The Lederle group have described an interesting new type of structure, *viz.*, the 17α : 21-epoxide (61), which is obtained by treatment of a 17α-hydroxy-20-(ethylene ketal) 21-toluene-*p*-sulphonate (60) with alkali, followed by hydrolysis of the ketal with dilute sulphuric acid.[132]



Ts = $Me{\cdot}C_6H_4{\cdot}SO_2$. R = $-O{\cdot}CH_2{-}CH_2{\cdot}O-$ or =O.

The discovery that 21-hydroxy-5β-pregnane-3 : 20-dione (as its hemisuccinate) is an anæsthetic [138d] may portend a new development in applied steroid chemistry.

Other work on the D-ring and the side-chain includes studies of (i) the formation of 17β-amines by Beckmann rearrangement of 20-oximes,[133] (ii) a variant of the 20-enol acetate epoxide procedure for preparing 17α-hydroxy-20-ketones, using $\Delta^{17(20)}$-20-acetamido-compounds,[134] (iii) reduction of 21-benzylidene-20-ketones with metal hydrides in various conditions,[135] (iv) reactions of 17β-toluene-*p*-sulphonates,[136] (v) cleavage of

[131] L. H. Sarett, R. M. Lukes, R. E. Beyler, G. I. Poos, W. F. Johns, and J. M. Constantin, *J. Amer. Chem. Soc.*, 1952, **74**, 4974 (*Ann. Reports*, 1952, **49**, 192); A. Ercoli, P. de Ruggieri, and D. Della Morte, *Gazzetta*, 1955, **85**, 628; A. Ercoli and P. de Ruggieri, *ibid.*, p. 639.

[132] W. S. Allen, S. Bernstein, M. Heller, and R. Littell, *J. Amer. Chem. Soc.*, 1955, **77**, 4784. For a simple analogue, see J. R. Marshall and J. Walker, *J.*, 1952, 467.

[133] J. Schmidt-Thomé, *Chem. Ber.*, 1955, **88**, 895.

[134] H. Ruschig, W. Fritsch, J. Schmidt-Thomé, and W. Haede, *ibid.*, p. 883.

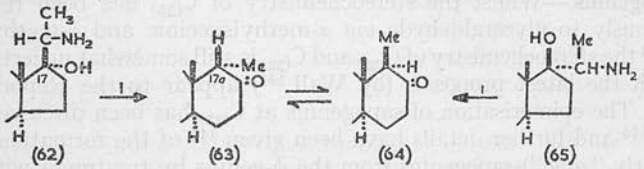
[135] E. P. Oliveto, C. Gerold, and E. B. Hershberg, *J. Amer. Chem. Soc.*, 1954, **76**, 6111.

[136] O. S. Madayeva, *Zhur. obshchei Khim.*, 1955, **25**, 1427; O. S. Madayeva and N. P. Babanova, *ibid.*, p. 1950.

16 : 17-disubstituted compounds with alkali in air,[137] and (vi) 21(and 22)-amino-compounds as potential fungicides.[138]

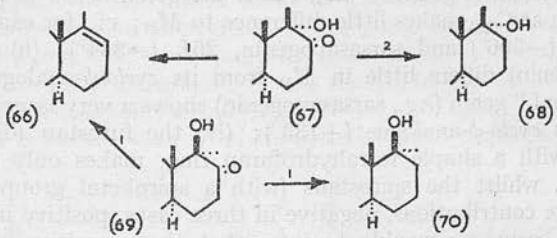
New compounds isolated from human urine include 3α : 17α : 21-trihydroxy-5β-pregnan-20-one,[138a] four adrenocortical metabolites with glycerol side-chains [17α : 20α(or β) : 21-triols],[138b] and the glucosuronidate of "tetrahydrocortisone" (3α : 17α : 21-trihydroxy-5β-pregnane-11 : 20-dione).[138c]

D-Homosteroids.—Interest in these compounds has increased during the past year. Ring enlargement [139] of isomeric 20-amino-17-hydroxysteroids (62, 65) has provided stereospecific routes to the two epimeric D-homo-17*a*-methyl-17-ketones (63, 64) involving migration of the 13 : 17-bond to $C_{(20)}$. The 17*a*β(e)-epimer (64) predominates in the equilibrium mixture of (63) and (64).



Reagent : 1, HNO_2.

Demjanow rearrangement [126] of either 17β-aminomethyl-17α-hydroxy- or 17α-aminomethyl-17β-hydroxy-compounds yields the 17*a*- and 17-oxo-D-homosteroids in the proportions 6 : 1.



Reagents : 1, Wolff–Kishner. 2, Indirect.

Turner, Heusser, and their colleagues [140] have correlated the 17*a*-hydroxy-17*a*-methyl-17-oxo-D-homo-compounds (67, 69) with the corresponding

[137] J. C. Touchstone, W. H. Elliott, S. A. Thayer, and E. A. Doisy, *J. Amer. Chem. Soc.*, 1955, **77**, 3562.

[138] R. A. Micheli and C. K. Bradsher, *ibid.*, p. 4788; H. L. Herzog, C. C. Payne, and E. B. Hershberg, *ibid.*, p. 5324.

[138a] J. P. Rosselet, L. Overland, J. W. Jailer, and S. Lieberman, *Helv. Chim. Acta*, 1954, **37**, 1933.

[138b] D. K. Fukushima, N. S. Leeds, H. L. Bradlow, T. H. Kritchevsky, M. B. Stokem, and T. F. Gallagher, *J. Biol. Chem.*, 1955, **212**, 449. This paper contains an interesting example of the use of the neighbouring-group effect in the conversion of a 20β-hydroxysteroid into its 20α-isomer.

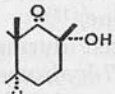
[138c] J. J. Schneider, M. L. Lewbart, P. Levitan, and S. Lieberman, *J. Amer. Chem. Soc.*, 1955, **77**, 4184.

[138d] G. D. Laubach, S. Y. P'an, and H. W. Rudel, *Science*, 1955, **122**, 78.

[139] F. Ramirez and S. Stafiej, *J. Amer. Chem. Soc.*, 1955, **77**, 134; 1956, **78**, 644; *Chem. and Ind.*, 1955, 1180.

[140] R. B. Turner, R. Anliker, R. Helbling, J. Meier, and H. Heusser, *Helv. Chim. Acta*, 1955, **38**, 411.

deoxo-compounds (68, 70). Consideration of other examples leads the authors to suggest the generalisation that whilst Wolff–Kishner reduction of α-ketols with an *axial* hydroxyl group leads almost entirely to unsaturated compounds (*e.g.*, 66), similar reduction of α-ketols with *equatorial* hydroxyl groups gives the unsaturated compound and the (deoxo)alcohol (*e.g.*, 70) in comparable yields.


(71)

Studies by the Sloan–Kettering and the Merck group [141] on the D-homo-ketols obtained from 17α-hydroxy-20-ketones have shown that, whilst bases give the 17*a*-hydroxy-17*a*-methyl-17-ketones, aluminium-*tert*.-butoxide or boron trifluoride gives chiefly the 17α-hydroxy-17β-methyl-17*a*-ketone (71). Other work on D-homo-compounds has been discussed on p. 218.

Sapogenins.—Whilst the stereochemistry of $C_{(25)}$ has been related unambiguously to glyceraldehyde *via* α-methylsuccinic and α-methylglutaric acids,[142] the stereochemistry of $C_{(20)}$ and $C_{(22)}$ is still somewhat uncertain,[143, 144] although the latest proposals (by Wall [144]) appear to the Reporter to be sound. The epimerisation of sapogenins at $C_{(25)}$ has been discussed by two groups,[145] and further details have been given [146] of the formation of *cyclo*-ψ(formerly "*ana*")-sapogenins from the ψ-genins by treatment with a trace of acid.

Wall [144, 147] has discussed the stereochemistry of $C_{(20)}$ and $C_{(22)}$ with reference to stability and reaction mechanisms for interconversion, and has for the first time laid stress on molecular rotation (M_D) differences between the different series of genins. (M_D values are given below in parentheses.) (i) Isomerism at $C_{(25)}$ makes little difference to M_D; cf., for example, smilagenin, 25*D* (−306°) and sarsasapogenin, 25*L* (−354°); (ii) an *iso*genin (*e.g.*, smilagenin) differs little in M_D from its *cyclo*-ψ-analogue (−248°); (iii) a "normal" genin (*e.g.*, sarsasapogenin) shows a very large difference in M_D from its *cyclo*-ψ-analogue (+133°); (iv) the furostan (dihydrogenin) side-chain, with a simple tetrahydrofuran ring, makes only a small M_D contribution, whilst the spirostans (with a spiroketal grouping at $C_{(22)}$) all show large contributions, negative in three cases, positive in the fourth. It therefore seems reasonable to infer that these positive and negative contributions are related to opposite configurations at $C_{(22)}$. The known configurations of all four isomers at $C_{(25)}$ and their stabilities then led Wall to suggest the formulæ (72—75). It may be noted that (73) is the only compound with an axial methyl group at $C_{(25)}$, and that the *cyclo*-ψ-compounds (74 and 75), which are both formed from the ψ-genins in very mild

[141] N. L. Wendler, D. Taub, D. K. Fukushima, and S. Dobriner, *Chem. and Ind.*, 1955, 1259; N. L. Wendler and D. Taub, *ibid.*, p. 505; D. K. Fukushima, S. Dobriner, M. S. Heffler, T. H. Kritchevsky, F. Herling, and G. Roberts, *J. Amer. Chem. Soc.*, 1955, **77**, 6585.

[142] *Ann. Reports*, 1953, **50**, 219; V. H. T. James, *J.*, 1955, 637; I. Scheer, R. E. Kostic, and E. Mosettig, *J. Amer. Chem. Soc.*, 1955, **77**, 641.

[143] J. B. Ziegler, W. E. Rosen, and A. C. Shabica, *ibid.*, p. 1223; M. E. Wall, S. Serota, and C. R. Eddy, *ibid.*, p. 1230.

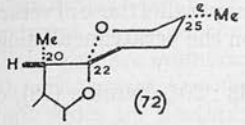
[144] M. E. Wall, *Experientia*, 1955, **11**, 340.

[145] R. K. Callow and V. H. T. James, *J.*, 1955, 1671; M. E. Wall, S. Serota, and L. P. Witnauer, *J. Amer. Chem. Soc.*, 1955, **77**, 3086.

[146] R. K. Callow, D. H. W. Dickson, J. Elks, R. M. Evans, V. H. T. James, A. G. Long, J. F. Oughton, and J. E. Page, *J.*, 1955, 1966.

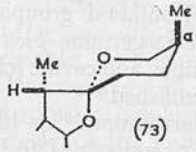
[147] M. E. Wall and H. A. Walens, *J. Amer. Chem. Soc.*, 1955, **77**, 5661; R. K. Callow and V. H. T. James, *Chem. and Ind.*, 1956, 112.

acid conditions by ring closure between $C_{(25)}$ and $C_{(22)}$, both have equatorial methyl groups at $C_{(25)}$.



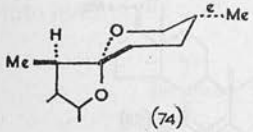
(72)

Smilagenin
(20α : 22*a* : 25*D*)



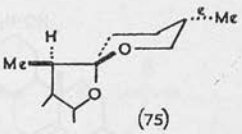
(73)

Sarsasapogenin
(20α : 22*a* : 25*L*)



(74)

cyclo-ψ-Smilagenin
(20β : 22*a* : 25*D*)



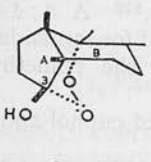
(75)

cyclo-ψ-Sarsasapogenin
(20β : 22*b* : 25*L*)

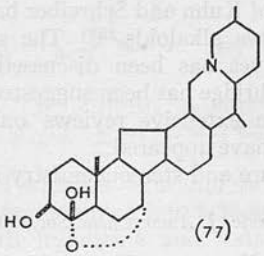
Whilst 20α-genins are stable to chromic acid in acetic acid at 15°, the 20β-genins are attacked in these conditions,[147] giving acidic intermediates (which on treatment with alkali yield Δ^{16}-20-ketones) and, in some cases, compounds containing an additional hydroxyl group (possibly at position 20).

Work on digitogenin [108–110] is reported on p. 217. Samogenin is 5β : 22*a* : 25*D*-spirostan-2β : 3β-diol; markogenin is its 25*L*-isomer; mexogenin is 12-oxosamogenin.[47] Other work includes improvements in the standard degradation procedure for conversion of a ψ-genin into a 20-oxosteroid,[148] isolation of two new Δ^5-12-oxosapogenins (gentrogenin and correllogenin) [149] and a Δ^5-3β : 19-diol (ruscogenin),[150] and studies in the hecololactone (13-hydroxy-12 : 13-*seco*-12-oic lactone) series.[151]

Alkaloids.—Further work from the Zurich school (reported briefly last

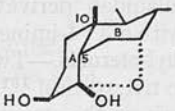


(76)



(77)

(+2 *sec.*-hydroxy- and 3-*tert.*-hydroxy-groups)



(perspective of hemi-ketal)

year) has settled some points in the stereochemistry of the cevine alkaloids.[152] Oxidation of the 3 : 4-ketol system in cevine to a 3 : 4-dione with bismuth

[148] M. E. Wall, H. E. Kenney, and E. S. Rothman, *J. Amer. Chem. Soc.*, 1955, **77**, 5665; A. F. B. Cameron, R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones, and A. G. Long, *J.*, 1955, 2807.

[149] H. A. Walens, S. Serota, and M. E. Wall, *J. Amer. Chem. Soc.*, 1955, **77**, 5196.

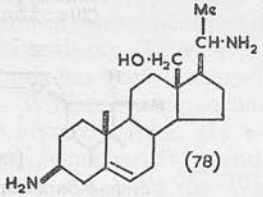
[150] C. Sannié and H. Lapin, *Bull. Soc. chim. France*, 1955, 1556.

[151] E. S. Rothman and M. E. Wall, *J. Amer. Chem. Soc.*, 1955, **77**, 2228.

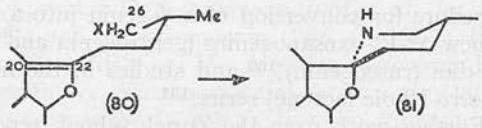
[152] M. V. Mijović, E. Sundt, E. Kyburz, O. Jeger, and V. Prelog, *Helv. Chim. Acta*, 1955, **38**, 231; F. Gautschi, O. Jeger, V. Prelog, and R. B. Woodward, *ibid.*, p. 296.

oxide in acetic acid is followed by benzilic acid type rearrangement, to gi the δ-lactone "cevinilic lactone" (76).[153] A cevane structure with 4α : 7α-hemiketal grouping (77) has been proposed for germine;[154] t germine–*iso*germine–*pseudo*germine isomerisations parallel those of veracevi cevagenine, and cevine (cf. ref. 152). A review on the veratrum alkaloids h been published.[155]

Holarrhimine[156] is 18-hydroxypregn-5-ene-3β : 20ξ-diamine (78), clos related to conessine (79).[156a]



Partial synthesis of tomatidine from ψ-25*L*-tigogenin[157] shows that t alkaloid must have a 25*L*-configuration, opposite to that in solasodi (as 81), which is obtained in a similar way from ψ-diosgenin (25*D*). T partial synthesis from a ψ-genin (80) involves the formation of a 26-phth imido-derivative *via* the 26-toluene-*p*-sulphonate and iodide, and th closure of ring F by treatment with hydrazine in ethanol. It is suggest that both 25*D* and 25*L* series have 20α : 22*a*-configurations.



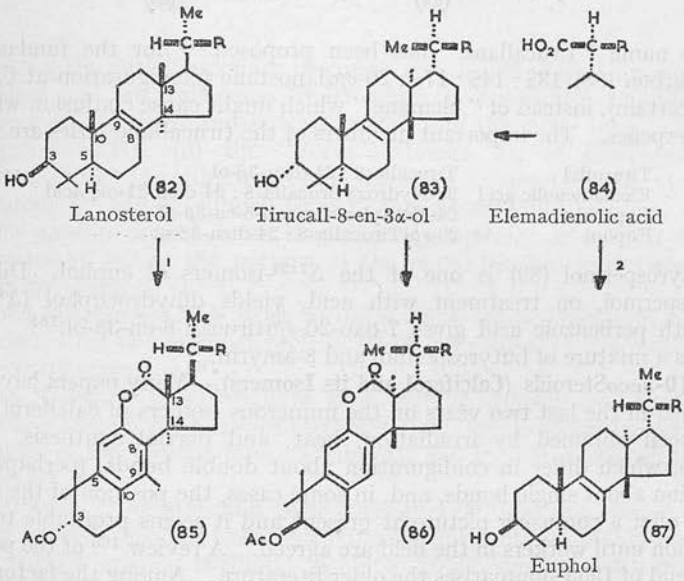
Papers from the schools of Kuhn and Schreiber have dealt with the gly side moiety of some *Solanum* alkaloids.[158] The stereochemistry of so isomeric solanidan derivatives has been discussed.[159] A 3 : 4-*seco*ster structure with a 3 : 4-imine bridge has been suggested for samandarine.[160]

Trimethyl-steroids.—Two extensive reviews on the trimethyl-steroi (tetracyclic triterpenes)[161] have appeared.

The work on the structure and stereochemistry of euphol and tirucall

[153] S. M. Kupchan and D. Lavie, *J. Amer. Chem. Soc.*, 1955, **77**, 683; S. M. Kupch *ibid.*, p. 686.
[154] S. M. Kupchan and C. R. Narayanan, *Chem. and Ind.*, 1955, 251; S. M. Kupch M. Fieser, C. R. Narayanan, L. F. Fieser, and J. Fried, *J. Amer. Chem. Soc.*, 1955, 5896.
[155] A. Stoll, *Gazzetta*, 1954, **84**, 1190.
[156] L. Labler, V. Cerny, and F. Šorm, *Chem. and Ind.*, 1955, 1119; V. Cerny F. Šorm, *Coll. Czech. Chem. Comm.*, 1955, **20**, 1473; L. Labler, V. Cerny, and F. Šo *ibid.*, p. 1484.
[156a] R. D. Haworth, *Chem. Soc. Special Publ.*, 1955, No. 3, 1.
[157] F. C. Uhle and J. A. Moore, *J. Amer. Chem. Soc.*, 1954, **76**, 6412.
[158] R. Kuhn, I. Low, and H. Trischmann, *Chem. Ber.*, 1955, **88**, 289, 1492, 1 K. Schreiber, *Angew. Chem.*, 1955, **67**, 127; *Chem. Tech.* (*Berlin*), 1955, **7**, 271.
[159] Y. Sato and H. G. Latham, jun., *Chem. and Ind.*, 1955, 444.
[160] C. Schöpf and D. Klein, *Chem. Ber.*, 1954, **87**, 1638.
[161] E. R. H. Jones and T. G. Halsall, *Fortschr. Chem. org. Naturstoffe*, 1955, 12, R. M. Gascoigne and J. J. H. Simes, *Quart. Rev.*, 1955, **9**, 328.

discussed in the Reports last year, has been extended by the schools of Jeger, Barton, and Warren.[162] These studies have now been crowned by the Zürich school [163] with an ingenious degradation of lanosterol (82) and elemadienolic acid (84) to *enantiomeric* acetoxyphenol lactones (85, 86), which demonstrates that the compounds of the elemadienolic acid–euphorbol–tirucallol series are epimeric with lanosterol at all four positions 13, 14, 17, and 20. The correlation depends on a sequence of reactions applied in the lanostane series by Barton and his colleagues,[163a] in which the asymmetry at $C_{(5)}$ and $C_{(10)}$ is destroyed in the aromatisation of ring B. In the elemadienolic acid series it was necessary first to convert the 21-carboxylic acid group into methyl (84→83).



R = ·$[CH_2]_2$·CH:CMe_2 or ·$[CH_2]_3$·$CHMe_2$.
1, Five stages. 2, By-product from the 3β-ol.

Correlation of euphol with the tirucallol series was achieved as follows. Elemadienolic acid was transformed into a 3β-acetoxy-21-oxo-Δ^8-compound which on reduction with hydrazine and sodium ethoxide gave tirucall-8-en-3β-ol [as (83) with 3β-OH] as the principal product, and euphol (87) as a by-product.[163] Euphol must therefore have the *same* configuration at $C_{(17)}$ as tirucallol and the *opposite* configuration at $C_{(20)}$.

Further valuable evidence has recently been obtained by the South African workers; [164] they transformed tiruculla-8 : 24-diene and the

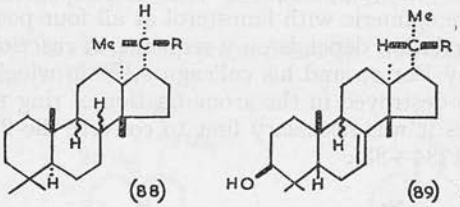
[162] Ref. 1, pp. 239—240; three references given in last year's Report are from the early months of 1955. See also J. B. Barbour, W. A. Lourens, F. L. Warren, and K. H. Watling, *J.*, 1955, 2194.

[163] E. Menard, H. Wyler, A. Hiestand, D. Arigoni, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, 1955, **38**, 1517.

[163a] C. S. Barnes, D. H. R. Barton, J. S. Fawcett, and B. R. Thomas, *J.*, 1952, 2339.

[164] F. L. Warren and K. H. Watling, *Chem. and Ind.*, 1956, 24.

analogous euphadiene by oxidation and partial Meystre–Miescher degradation into products carrying the 17-side chain $-CMe{:}CH{\cdot}CH{:}CPh_2$, in which the asymmetry of $C_{(20)}$ has been eliminated. The identity of the two products shows that the starting materials differed in stereochemistry only at $C_{(20)}$.



The name " tirucallane " has been proposed [163] for the fundamental hydrocarbon (88) 13α : 14β : 17α : 20-*epi*lanostane (configuration at $C_{(8)}$ and $C_{(9)}$ uncertain), instead of " elemane " which might cause confusion with the sesquiterpenes. The important members of the tirucallane series are :

Tirucallol	Tirucalla-8 : 24-dien-3β-ol
Elemadienolic acid	3α-Hydroxytirucalla-8 : 24-dien-21-oic acid
Euphorbol	24-Methylenetirucall-8-en-3β-ol
Euphol	20-*epi*Tirucalla-8 : 24-dien-3β-ol

Butyrospermol (89) is one of the $\Delta^{7:24}$-isomers of euphol. Dihydro butyrospermol, on treatment with acid, yields dihydroeuphol ($\Delta^7 \rightarrow \Delta^8$) and with perbenzoic acid gives 7-oxo-20-*epi*tirucall-8-en-3β-ol.[165] " Bas seol " is a mixture of butyrospermol and β-amyrin.

9: 10-*seco*Steroids (Calciferol and its Isomers).—Many papers have been published in the last two years on the numerous isomers of calciferol which have been obtained by irradiation, heat, and partial synthesis. These isomers, which differ in configuration about double bonds, (perhaps) con formation about single bonds, and, in some cases, the position of the doubl bonds, offer a confusing picture at present and it seems profitable to defe discussion until workers in the field are agreed. A review [166] of the positio at the end of 1953 summarises the older literature. Among the factors use as evidence for configurations are ultraviolet absorption (combined wit synthesis of simple reference compounds and/or theoretical arguments) an reactions with maleic anhydride.

The views of various schools are given in the references indicated.[167-17

[165] D. S. Irvine, W. Lawrie, A. S. McNab, and F. S. Spring, *Chem. and Ind.*, 19 626; M. C. Dawson, T. G. Halsall, E. R. H. Jones, G. D. Meakins, and P. C. Philli *ibid.*, p. 918.

[166] H. H. Inhoffen and K. Brückner, *Fortschr. Chem. org. Naturstoffe*, 1954, **11**, 8

[167] I. T. Harrison, B. Lythgoe, and S. Trippett, *J.*, 1955, 4016.

[168] F. Sondheimer and O. H. Wheeler, *Chem. and Ind.*, 1955, 714.

[169] E. A. Braude and O. H. Wheeler, *J.*, 1955, 320, 329.

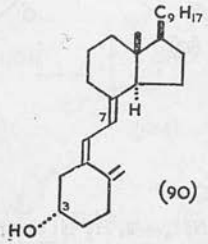
[170] L. Velluz, G. Amiard, and B. Goffinet, *Bull. Soc. chim. France*, 1955, 1341.

[171] H. H. Inhoffen, K. Brückner, G. F. Domagk, and H. M. Erdmann, *Chem. Be* 1955, **88**, 1415; H. H. Inhoffen, K. Brückner, K. Irmscher, and G. Quinkert, *ibi* p. 1424; H. H. Inhoffen, K. Brückner, R. Gründel, and G. Quinkert, *ibid.*, 1954, 8 1407; H. H. Inhoffen and G. Quinkert, *ibid.*, p. 1418; H. H. Inhoffen and J. Ka *ibid.*, p. 1589.

[172] E. Havinga, A. L. Koevoet, and A. Verloop, *Rec. Trav. chim.*, 1955, **74**, 1230.

[173] L. Velluz and G. Amiard, *Bull. Soc. chim. France*, 1955, 205; L. Velluz, G. Amia and B. Goffinet, *Compt. rend.*, 1955, **240**, 2076, 2326.

The configuration (90) of calciferol suggested by Lythgoe and his colleagues[167] agrees with that proposed on *X*-ray evidence seven years ago.[174]



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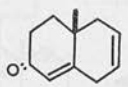


(91)

Among the new compounds discussed may be noted (i) the pre-calciferol of Velluz and his colleagues [170, 173] which is apparently the immediate precursor of calciferol and is transformed into the latter by a non-photochemical reaction, (ii) the "*trans*"-vitamins D_2 of Havinga [175] and Inhoffen,[176] (iii) the dihydrovitamin D_2-II of Schubert,[177] (iv, v) the *iso*-tachysterol [178] and *u*("umgelagertes")-tachysterol [171] of Inhoffen.

The Leiden school have employed isotopically labelled 7-dehydrocholesterol to follow the pattern of the photochemical conversion of the various provitamins D.[172]

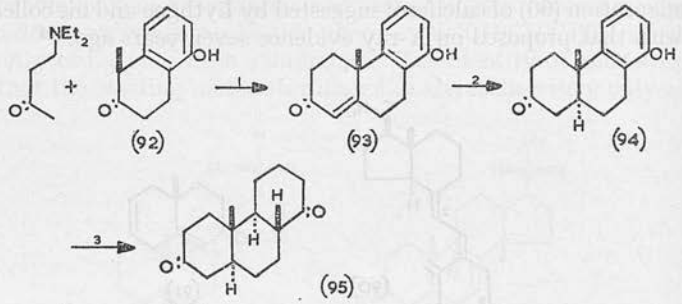
Total Synthesis.—New work includes many improvements in syntheses discussed in the two previous reviews.[179, 180] A general review by Cornforth has recently appeared.[181]

The Monsanto group [182] have given details of their total synthesis of cortisone based on the Harvard method (cf. ref. 180), and have described the resolution of the bicyclic intermediate (91) and its correlation with natural steroids.[183]

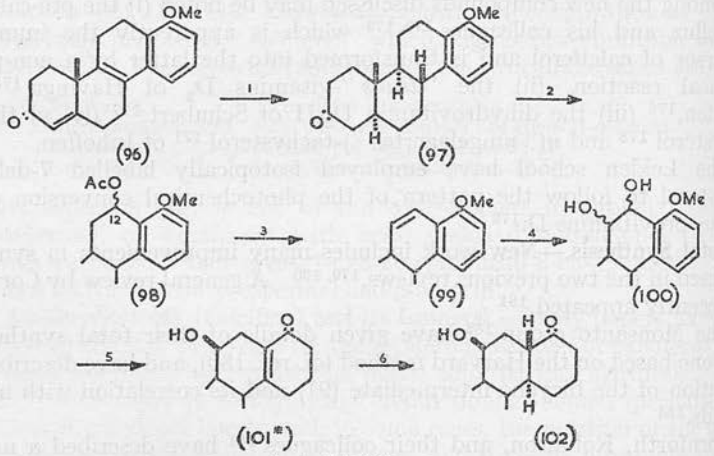
Cornforth, Robinson, and their colleagues [184] have described a number of lines of work directed towards a more elegant synthesis within the framework of their original method. A stereospecific synthesis of a *trans-anti-trans*-perhydrophenanthrene derivative takes the course (92—95).

The Wisconsin school [185] have extended their ingenious total synthesis of *epi*androsterone to prepare an 11-hydroxy-compound from the intermediate (96).

[174] D. Crowfoot and J. D. Dunitz, *Nature*, 1948, **162**, 608.
[175] A. Verloop, A. L. Koevoet, and E. Havinga, *Rec. Trav. chim.*, 1955, **74**, 1125.
[176] H. H. Inhoffen, J. F. Kath, and K. Brückner, *Angew. Chem.*, 1955, **67**, 276.
[177] K. Schubert, *Naturwiss.*, 1954, **41**, 231; *Biochem. Z.*, 1954, **326**, 132.
[178] H. H. Inhoffen, K. Brückner, and R. Gründel, *Chem. Ber.*, 1954, **87**, 1.
[179] *Ann. Reports*, 1952, **49**, 190.
[180] *Ibid.*, 1953, **50**, 219.
[181] J. W. Cornforth, "Progress in Organic Chemistry," ed. J. W. Cook, Butterworths, London, 1955, Vol. III, p. 1.
[182] L. B. Barkley, M. W. Farrer, W. S. Knowles, and H. Raffelson, *J. Amer. Chem. Soc.*, 1954, **76**, 5017.
[183] A. J. Speziale, J. A. Stephens, and Q. E. Thompson, *ibid.*, p. 5011; L. B. Barkley, M. W. Farrer, W. S. Knowles, H. Raffelson, and Q. E. Thompson, *ibid.*, p. 5014.
[184] A. R. Pinder and (Sir) R. Robinson, *J.*, 1955, 3341; J. W. Cornforth, O. Kauder, J. E. Pike, and (Sir) R. Robinson, *J.*, 1955, 3348.
[185] W. S. Johnson, R. Pappo, and A. D. Kemp, *J. Amer. Chem. Soc.*, 1954, **76**, 3353.



Reagents: 1, KOEt. 2, Li–NH_3. 3, H_2–Ni on the ketal.



* And Δ^{16}-isomer.

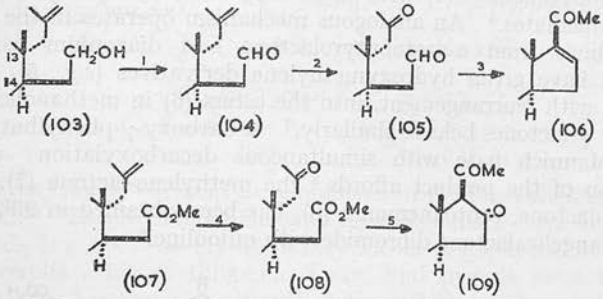
Reagents: 1, Li–EtOH–NH_3. 2, $Pb(OAc)_4$. 3, AcOH. 4, $H{\cdot}CO_3H$. 5, (*a*) Li–EtOH–NH_3; (*b*) H^+. 6, H_2–Pd. Then as in 11-deoxy series.

The introduction of the 11-hydroxy-group *via* 12-acetoxy-, Δ^{11}-, 11 : 12-diol groupings is noteworthy. Other papers from the same school deal with 18 : 19-bisnor-D-homotestosterone [186] and testosterone.[187]

A number of papers from the Merck laboratories have described in detail later stages in the total synthesis of cortisone [188] (cf. ref. 179) and its subsequent refinements,[189, 190] Two new methods of closing ring D are noteworthy. In the first,[189] oxidation of the primary alcohol (103) to an ald

[186] W. S. Johnson, H. C. Dehm, and L. J. Chinn, *J. Org. Chem.*, 1954, **19**, 670.
[187] W. S. Johnson, B. Bannister, R. Pappo, and J. E. Pike, *J. Amer. Chem. So* 1955, **77**, 817.
[188] G. E. Arth, G. I. Poos, R. M. Lukes, F. M. Robinson, W. F. Johns, M. Feur and L. H. Sarett, *J. Amer. Chem. Soc.*, 1954, **76**, 1715; W. F. Johns, R. M. Lukes, a L. H. Sarett, *ibid.*, 5026; G. I. Poos, R. M. Lukes, G. E. Arth, and L. H. Sarett, *ibi* p. 5031.
[189] G. I. Poos, W. F. Johns, and L. H. Sarett, *ibid.*, 1955, **77**, 1026.
[190] G. E. Arth, G. I. Poos, and L. H. Sarett, *ibid.*, p. 3834.

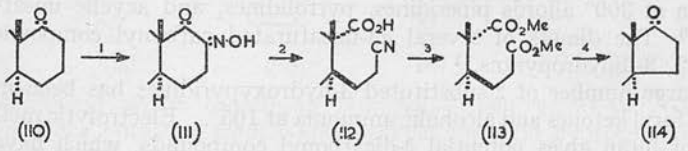
hyde is followed by removal of the 20-methylene group by known procedures; cyclisation of the ε-keto-aldehyde (105) with aqueous potassium hydroxide (free from oxygen) then yields a Δ^{16}-20-ketone (106). The second



Reagents: 1, CrO_3–pyridine. 2, (*a*) OsO_4, (*b*) HIO_4. 3, KOH. 4, as 1 and 2. 5, NaOMe.

method [190] starts from the methoxycarbonyl compound (107) corresponding to (103). Cyclisation of the ε-keto-ester (108) with sodium methoxide in benzene gives the 16 : 20-diketone (109), which can then be transformed into the Δ^{16}-20-ketone and the saturated 20-ketone.

The Ciba group,[191] continuing their total synthesis which has led to a D-homo-17*a*-ketone (110), have described a method of contracting a six-membered to a five-membered ring which involves ring-opening of a hydroxyimino-ketone (111).



Reagents: 1, C_5H_{11}·O·NO–Bu^{t}OK. 2, *p*-C_6H_4Me·SO_2Cl–NaOH. 3, (*a*) KOH; (*b*) CH_2N_2. 4, NaOMe.

An extensive series of papers by Nazarov and his colleagues [192] deals chiefly with bicyclic and tricyclic intermediates. Other work on polyhydrophenanthrenes has been reported.[193]

W. K.

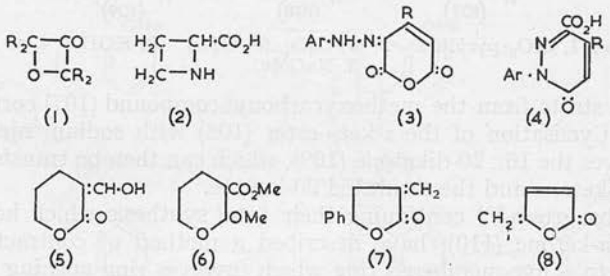
8. HETEROCYCLIC COMPOUNDS.

Small Rings.—The oxetanones (1; R = Me and Ph) have been made, the latter in 47% yield by autoxidation of *sym.*-tetraphenylacetone in acetic acid.[1] Azetidine-2-carboxylic acid (2) has been isolated from *Convallaria majalis* Lin., and its structure established by ring scissions.[2] Certain

[191] P. Wieland, G. Anner, and K. Miescher, *Helv. Chim. Acta*, 1953, **36**, 1803.
[192] I. N. Nazarov, L. D. Bergelson, I. V. Torgov, and S. N. Ananchenko, *Izvest. Akad. Nauk, S.S.S.R., Otdel. Khim. Nauk*, 1953, 889, and subsequent papers.
[193] C. A. Grob and O. Schindler, *Experientia*, 1954, **10**, 367; N. Chaudhuri and P. C. Mukharji, *Sci. and Cult.*, 1954, **19**, 463.
[1] B. L. Murr, G. B. Hoey, and C. T. Lester, *J. Amer. Chem. Soc.*, 1955, **77**, 4430; G. B. Hoey, D. O. Dean, and C. T. Lester, *ibid.*, p. 391.
[2] L. Fowden, *Nature*, 1955, **176**, 347.

β-lactones are formed surprisingly easily from β-hydroxy-acids in dilute acids.[3]

Simple Lactones, Furans, and Pyrans.—Glutaconic anhydrides form dihydro-oxopyridazines (4) with diazonium salts, hydrazones (3) probably being intermediates.[4] An analogous mechanism operates in the formation of pyrazolines from α-acetobutyrolactone and diazonium compounds.[5] δ-Lactones have given hydroxymethylene derivatives (*e.g.*, 5), which are converted, with rearrangement, into the esters (6) in methanolic hydrogen chloride;[6] γ-lactones behave similarly.[7] α-Carboxy-γ-phenylbutyrolactone forms a Mannich base with simultaneous decarboxylation; exhaustive methylation of the product affords[8] the methylene-lactone (7). Another methylene-lactone, protoanemonin (8), has been obtained in 99% yield by treating α-angelicalactone dibromide with quinoline.[9]

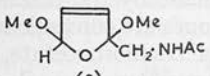


Passage of mixtures of 2-alkyltetrahydropyrans and primary amines over alumina at 300° affords piperidines, pyrrolidines, and acyclic unsaturated bases.[10] The dimers of several αβ-unsaturated carbonyl compounds are 2-acyl-2 : 3-dihydropyrans.[11]

A large number of 2-substituted 3-hydroxypyridines has been made[12] from 2-furyl ketones and alcoholic ammonia at 165°. Electrolytic methoxylation of furan gives potential δ-dicarbonyl compounds, which have been used in a number of interesting syntheses.[13, 14, 15] For example,[13] electrolysis of 2-acetamidomethylfuran in methanol gives the dihydrodimethoxyfuran (9), which is converted into 3-hydroxypyridine (93%) in N-hydro-

[3] J. H. Wotiz and J. S. Matthews, *J. Org. Chem.*, 1955, **20**, 155.
[4] R. H. Wiley and C. H. Jarboe, *J. Amer. Chem. Soc.*, 1955, **77**, 403; R. H. Wiley and H. G. Ellert, *ibid.*, p. 5187.
[5] G. F. Duffin and J. D. Kendall, *J.*, 1955, 3470.
[6] F. Korte and H. Machleidt, *Chem. Ber.*, 1955, **88**, 136, 1676.
[7] *Idem*, *ibid.*, p. 1685.
[8] E. E. van Tamelen and S. R. Bach, *J. Amer. Chem. Soc.*, 1955, **77**, 4683.
[9] C. Grundmann and E. Kober, *ibid.*, p. 2332.
[10] H. P. Richards and A. N. Bourns, *Canad. J. Chem.*, 1955, **33**, 1433.
[11] J. Matti and M. Perrier, *Bull. Soc. chim. France*, 1955, 525; J. Dreux, *ibid.*, p. 521; M. Delépine, G. Amiard, M. Badoche, P. Compagnon, A. Horeau, J. Jacques, and A. Willemart, *Ann. Chim.*, 1955, **10**, 5; R. H. Hall, *J.*, 1954, 4303.
[12] W. Gruber, *Chem. Ber.*, 1955, **88**, 178.
[13] N. Clauson-Kaas, N. Elming, and Z. Tyle, *Acta Chem. Scand.*, 1955, **9**, 1.
[14] J. T. Nielsen, N. Elming, and N. Clauson-Kaas, *ibid.*, p. 9; N. Clauson-Kaas and P. Nedenskov, *ibid.*, p. 14; P. Nedenskov, N. Elming, J. T. Nielsen, and N. Clauson-Kaas, *ibid.*, p. 17; N. Clauson-Kaas and P. Nedenskov, *ibid.*, p. 27; J. T. Nielsen, N. Elming, and N. Clauson-Kaas, *ibid.*, p. 30; J. T. Nielsen, N. Clauson-Kaas, and P. Dietrich, *ibid.*, p. 182.
[15] N. Elming and N. Clauson-Kaas, *ibid.*, p. 23.

chloric acid. Pyridoxine has been made by a similar method in 76% yield from a suitably substituted furan.[15]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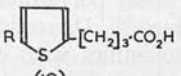
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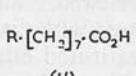
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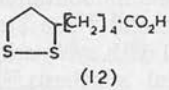
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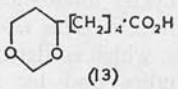
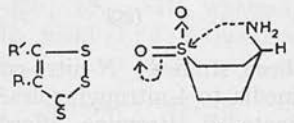
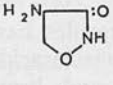

(13)

Sulphur Compounds.—Recent developments in thiophen chemistry have been reviewed.[16] Several long-chain acids have been made from substituted thiophen acids (*e.g.*, 10 ⟶ 11) by Raney nickel desulphurisation.[17] Di- and tri-carboxylic acids of thiophen, furan, and pyrrole have been described.[18] There has been continued interest [19,20] in the synthesis of α-lipoic (6-thioctic) acid (12); one ingenious synthesis [20] employs the intermediate (13), made by Prins condensation of formaldehyde with hept-6-enoic acid. 1 : 2-Dithiole-3-thiones (14) appear to be fairly stable; they have been made from β-oxo-esters and phosphorus pentasulphide,[21] and by heating α-methylstilbenes [22] or cumenes [23] with sulphur. The dioxide (15) is a much weaker base than 4-aminotetrahydrothiopyran, probably because of the intramolecular interaction shown.[24]


(14) (15)


(16)

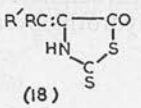

(17)

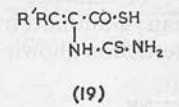
Five-membered Rings containing Nitrogen.—Good yields of *N*-alkylpyrrolidones are obtained from γ-alkylbenzylamino-acid hydrochlorides in boiling acetic anhydride, debenzylation occurring simultaneously.[25] Reductive condensation of hydroxyiminomalonic ester with β-dicarbonyl compounds, effected with zinc dust in acetic acid, provides a valuable new synthesis of substituted pyrrole-2-carboxylic esters.[26] Several relatively simple pyrroles form stable crystalline salts with hydrogen bromide in dry ether.[27]

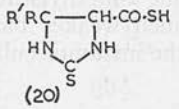
[16] F. F. Nord, A. Vaitiekunas, and L. J. Owen, *Fortschr. chem. Forsch.*, 1955, **3**, 309—333.
[17] M. Sy, *Bull. Soc. chim. France*, 1955, 1175; G. M. Badger, H. J. Rodda, and W. H. F. Sasse, *J.*, 1954, 4162.
[18] R. G. Jones, *J. Amer. Chem. Soc.*, 1955, **77**, 4069, 4163.
[19] L. J. Reed and C. Niu, *ibid.*, p. 416; E. Walton, A. F. Wagner, F. W. Batchelor, L. H. Peterson, F. W. Holly, and K. Folkers, *ibid.*, p. 5144.
[20] E. A. Braude, R. P. Linstead, and K. H. R. Wooldridge, *Chem. and Ind.*, 1955, 508.
[21] L. Legrand and N. Lozac'h, *Bull. Soc. chim. France*, 1955, 79; J. Teste and N. Lozac'h, *ibid.*, p. 437.
[22] J. Schmitt and M. Suquet, *ibid.*, p. 84.
[23] E. K. Fields, *J. Amer. Chem. Soc.*, 1955, **77**, 4255.
[24] C. Barkenbus and J. A. Wuellner, *ibid.*, p. 3866.
[25] M. W. Gittos and W. Wilson, *J.*, 1955, 2371.
[26] G. G. Kleinspehn, *J. Amer. Chem. Soc.*, 1955, **77**, 1546.
[27] R. J. Stedman and S. F. MacDonald, *Canad. J. Chem.*, 1955, **33**, 468.

2-Aryloxazolines are obtained from β-azido-alcohols and aromati aldehydes in concentrated sulphuric acid.[28] The chemistry of oxazol-5-on has been reviewed,[29] and linear polyamides made by the reaction of 2:2 bis(oxazolones) with diamines.[30] Oxazolid-2-ones are conveniently made b heating substituted ethanolamines with ethyl trichloroacetate, chlorofor being formed simultaneously; whilst γ-aminopropanols similarly give th six-membered cyclic analogues, δ-aminobutanols behave differently an afford pyrrolidines.[31] The *iso*oxazolid-2-one structure (16) for the anti biotic oxamycin, which is identical with *cyclo*serine, has been established b degradative studies and by total synthesis.[32] 2-Aminothiazole hydr chloride can be made in 91% yield from thiourea and 2-chloromethyl-1:3 dioxolan (17), which is readily accessible.[33] The infrared absorption pro perties of thiazolines have been recorded,[34] and it has been found tha thiazol-5-ones, obtained by heating α-thioacylamino-acids with acet anhydride, resemble oxazolones in reactions with carbonyl compounds an with amines.[35]

A series of novel glyoxaline derivatives (20) has been made, in additi to previously recognised products, by treating 4-alkylidene-2-thiothiazoli 5-ones (18) with ammonia, thioamides (19) being probable intermediates.[36]


(18)

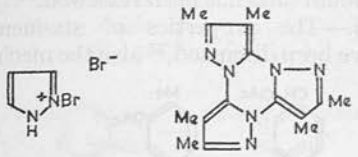

(19)


(20)

The nitration of pyrazoles has been studied; *N*-nitro-compounds a formed first, and rearrange in acid media to 4-nitropyrazoles.[37] Pyrazo are easily iodinated[38] and brominated.[39] Bromine affords crystall adducts (*e.g.*, 21); in the presence of iron powder, 4-bromo-, 3:4-dibrom and 3:4:5-tribromo-pyrazoles are obtained. Several condensation pr ducts are formed from pyrazoles and aqueous sodium hypobromite; f example, the complex compound (22), m. p. 278°, is one of the produ from 3:4-dimethylpyrazole.[39] The formation of furoxans (23) from a methyl ketones and nitric acid probably involves[40] dimerisation of int mediate nitrile oxides (24).

[28] J. H. Boyer and J. Hamer, *J. Amer. Chem. Soc.*, 1955, **77**, 951.
[29] E. Baltazzi, *Quart. Rev.*, 1955, **9**, 150.
[30] C. S. Cleaver and B. C. Pratt, *J. Amer. Chem. Soc.*, 1955, **77**, 1544, 1541.
[31] G. Y. Lesher and A. R. Surrey, *ibid.*, p. 636.
[32] F. A. Kuehl, F. J. Wolf, N. R. Trenner, R. L. Peck, E. Howe, B. D. Hunnew G. Downing, E. Newstead, R. P. Buhs, I. Potter, R. Ormond, J. E. Lyons, L. Cha and K. Folkers, *ibid.*, p. 2344; P. H. Hidy, E. B. Hodge, V. V. Young, R. L. Har G. A. Brewer, W. F. Phillips, W. F. Runge, H. E. Staveley, A. Pohland, H. Boaz, H. R. Sullivan, *ibid.*, p. 2345; C. H. Stammer, A. N. Wilson, F. W. Holly, and Folkers, *ibid.*, p. 2346.
[33] M. J. Astle and J. B. Pierce, *J. Org. Chem.*, 1955, **20**, 178.
[34] W. Otting and F. Drawert, *Chem. Ber.*, 1955, **88**, 1469.
[35] J. B. Jepson, A. Lawson, and V. D. Lawton, *J.*, 1955, 1791.
[36] F. P. Doyle, D. O. Holland, and J. H. C. Nayler, *J.*, 1955, 2265.
[37] R. Hüttel, F. Büchele, and P. Jochum, *Chem. Ber.*, 1955, **88**, 1577; R. Hü and F. Büchele, *ibid.*, p. 1586.
[38] R. Hüttel, O. Schäfer, and P. Jochum, *Annalen*, 1955, **593**, 200.
[39] R. Hüttel, H. Wagner, and P. Jochum, *ibid.*, p. 179.
[40] H. R. Snyder and N. E. Boyer, *J. Amer. Chem. Soc.*, 1955, **77**, 4233.

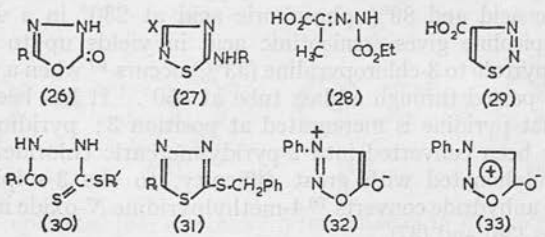
Several 1 : 2 : 4-oxadiazoles have been made by heating amidoximes with acetic anhydride or benzoyl chloride,[41] and 1 : 3 : 4-oxadiazoles (25) from acylhydrazines and orthoesters, *via* alkoxymethylenehydrazides.[42] 1 : 3 : 4-Oxadiazol-2-ones (26) are best made from hydrazides and carbonyl chloride,



(21) (22) (23) (25)

(24) Ar·CO·C:N→O

but they can be obtained by treating *N*-acyl-*N'*-chloroureas with sodium carbonate; the products undergo ring scission with amines, yielding semicarbazides and finally hydrazides.[43] Substituted 1 : 2 : 4-thiadiazoles (27; X = OR' and NR_2') are obtained from *O*-alkylureas[44] or *NN*-dialkylguanidines[45] by *N*-chlorination and reaction with sodium thiocyanate; and 5-alkylamino-3-amino-compounds (27; X = NH_2) from *N*-alkyl-*N'*-amidinothioureas and hydrogen peroxide in aqueous ethanol.[46] The reaction of thionyl chloride with certain acylhydrazones gives photosensitive 1 : 2 : 3-thiadiazoles (*e.g.*, 28 ⟶ 29), which thus become readily accessible.[47] The acids (30; R' = H) are cyclised in alkali to 1 : 3 : 4-oxadiazoles (25; R' = SH), whereas the corresponding benzyl esters (30; R' = CH_2Ph) yield 1 : 3 : 4-thiadiazoles (31) in concentrated sulphuric acid.[48]



(26) (27) (28) (29)

(30) (31) (32) (33)

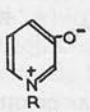
Current usage of the term " mesoionic " has been strongly criticised.[49] It has been stressed[50] that mesoionic compounds have true benzenoid aromatic structures, as seen in the betaine formulations (32) or (preferably) (33) for *N*-phenylsydnone. If the description " mesoionic " is retained, it should be applicable to all mesomeric aromatic betaines, including such compounds as (34) and tropone. Acid hydrolysis of alkylsydnones has been

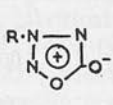
[41] K. Clarke, *J.*, 1954, 4251.
[42] C. Ainsworth, *J. Amer. Chem. Soc.*, 1955, **77**, 1148.
[43] A. Stempel, J. Zelauskas, and J. A. Aeschlimann, *J. Org. Chem.*, 1955, **20**, 412.
[44] J. Goerdeler and F. Bechlars, *Chem. Ber.*, 1955, **88**, 843.
[45] J. Goerdeler and M. Willig, *ibid.*, p. 1071.
[46] F. Kurzer, *J.*, 1955, 1, 2288.
[47] C. D. Hurd and R. I. Mori, *J. Amer. Chem. Soc.*, 1955, **77**, 5359.
[48] R. W. Young and K. H. Wood, *ibid.*, p. 400.
[49] A. R. Katritzky, *Chem. and Ind.*, 1955, 521, 1391.
[50] W. Baker and W. D. Ollis, *ibid.*, p. 910; T. I. Bieber, *ibid.*, p. 1055; W. J. O. Thomas, *ibid.*, p. 533.

used for the preparation of substituted hydrazines; [51, 52] 3-3′-pyridylsydnone is reversibly phototropic, the normal colourless form becoming deep blue in sunlight.[52] A new series of mesoionic compounds (35) has been made from alkylsemicarbazides and nitrous acid.[53]

The extensive chemistry of tetrazolium salts has been reviewed.[54]

Six-membered Nitrogenous Rings.—The properties of six-membered aromatic rings containing nitrogen have been discussed,[55] also the mechanism


(34)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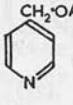
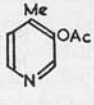
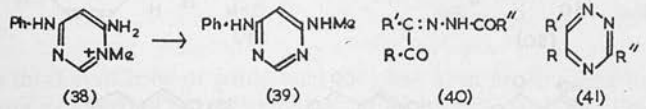

(36)


(37)

of nucleophilic substitution in these and related compounds.[56] Passing mixtures of alkyl or aryl cyanides or cyanogen with 1 : 3-dienes over alumina at 400° affords useful quantities of substituted pyridines; [57] and 2-2′-pyridylethylamines are conveniently made by the addition of secondary amines to 2-vinylpyridine.[58] Results are now available on the basic strengths and ultraviolet-light absorptions of a large number of alkyl-,[59] halogeno-,[60] and 3-hydroxy-pyridines [61] and pyridine *N*-oxides.[62] Large α-substituents in pyridine markedly hinder quaternary salt formation.[63] Oxidation of gaseous alkylpyridines with air at 380° over mixed vanadium-molybdenum oxides gives mainly pyridine-aldehydes.[64] Moderate yields of pyridinecarboxylic acids are obtained from alkylpyridines and selenium dioxide, but 3-methyl groups are not attacked.[65] Dilute nitric acid is a promising reagent for the oxidation of alkylpyridines; [66] thus, with a mixture of 10% nitric acid and 89% phosphoric acid at 230° in a stainless-steel autoclave, γ-picoline gives *iso*nicotinic acid in yields up to 95%. Ring expansion of pyrrole to 3-chloropyridine (33%) occurs [67] when a mixture with chloroform is passed through a glass tube at 550°. It has been known for some time that pyridine is mercurated at position 3; pyridine-2-sulphinic acid has now been converted into 2-pyridylmercuric chloride.[68] Pyridine *N*-oxide is sulphonated with great difficulty, to the 3-sulphonic acid.[69] Boiling acetic anhydride converts [70] 4-methylpyridine *N*-oxide into a mixture of the acetates (36) and (37).

[51] J. Fugger, J. M. Tien, and I. M. Hunsberger, *J. Amer. Chem. Soc.*, 1955, **77**, 1843.
[52] J. M. Tien and I. M. Hunsberger, *Chem. and Ind.*, 1955, 119.
[53] J. H. Boyer and F. C. Canter, *J. Amer. Chem. Soc.*, 1955, **77**, 1280.
[54] A. W. Nineham, *Chem. Rev.*, 1955, **55**, 355—483.
[55] A. Albert, *Chem. Soc. Special Publ.* No. 3, 1955, p. 124.
[56] N. B. Chapman, *ibid.*, p. 155.
[57] G. J. Jang and W. J. H. McCulloch, *J. Amer. Chem. Soc.*, 1955, **77**, 3014, 1343.
[58] H. E. Reich and R. Levine, *ibid.*, p. 4913.
[59] H. C. Brown and X. R. Mihm, *ibid.*, p. 1723.
[60] H. C. Brown and D. H. McDaniel, *ibid.*, p. 3752.
[61] D. E. Metzler and E. E. Snell, *ibid.*, p. 2431.
[62] H. H. Jaffé and G. O. Doak, *ibid.*, p. 4441; H. Jaffé, *ibid.*, p. 4451.
[63] H. C. Brown and A. Cahn, *ibid.*, p. 1715.
[64] W. Mathes and W. Sauermilch, *Chem. Ber.*, 1955, **88**, 1276.
[65] D. Jerchel, E. Bauer, and H. Hippchen, *ibid.*, p. 156.
[66] E. B. Bengtsson, *Acta Chem. Scand.*, 1955, **9**, 832.
[67] H. L. Rice and T. E. Londergan, *J. Amer. Chem. Soc.*, 1955, **77**, 4678.
[68] C. D. Hurd and C. J. Morrissey, *ibid.*, p. 4658.
[69] H. S. Mosher and F. J. Welch, *ibid.*, p. 2902.
[70] J. A. Berson and T. Cohen, *ibid.*, p. 1281.

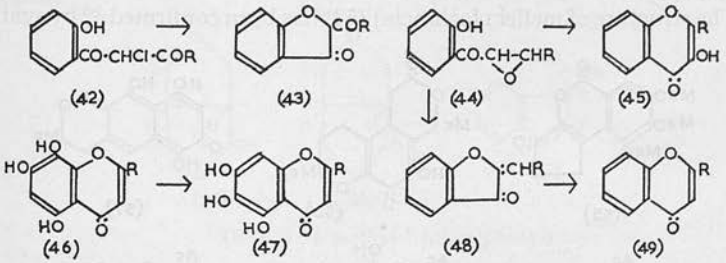
The rearrangement [71] of 1-methylpyrimidinium compounds, *e.g.*, (38) ⟶ (39), recalls several other rearrangements in the heterocyclic field.[72] Ultraviolet-light absorption studies show that 2- and 4-hydroxypyrimidines exist largely in the lactam form in solution; these compounds afford chiefly *N*-methyl derivatives with diazomethane.[73] A series of 1 : 2 : 4-triazines (41) has been made by treating α-diketone acylhydrazones (40) with ammonia.[74] 1 : 3 : 5-Triazine can be made in fair yields by heating form-



(38) (39) (40) (41)

amidine hydrochloride with bases; it is a powerful noble-metal catalyst poison.[75]

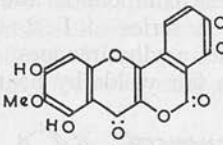
Condensed Ring Systems. Naturally occurring Oxygen Ring Compounds. —Some of these compounds are discussed in a recent monograph.[76] The Auwers synthesis of 2-acylcoumaran-3-ones (43) from *o*-acyloxy-ω-chloroacetophenones probably involves intermediates (42) formed by a Baker–Venkataraman transformation.[77] Fair yields of flavonols (45) are obtained from ω-chloro-*o*-hydroxyacetophenones in cold ethanolic potassium hydroxide, and 2-arylidenecoumaran-3-ones (aurones) (48) are formed at higher



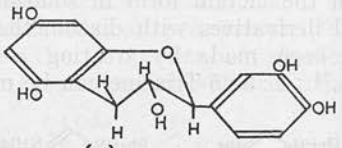
temperatures; epoxides (44) are likely intermediates in this condensation.[78] Aurones (48) undergo ring expansion to flavones (49) in ethanolic potassium cyanide [79] and give either aurone epoxides or flavonols (45) with alkaline hydrogen peroxide.[80] Rearrangement of 2-acylcoumarone oxime toluene-*p*-sulphonates is a convenient route to chromonols (especially) and flavonols;

[71] H. C. Carrington, F. H. S. Curd, and D. N. Richardson, *J.*, 1955, 1858.
[72] *Ann. Reports*, 1953, **50**, 238.
[73] D. J. Brown, E. Hoerger, and S. F. Mason, *J.*, 1955, 211.
[74] R. Metze, *Chem. Ber.*, 1955, **88**, 772.
[75] C. Grundmann, H. Schröder, and W. Ruske, *ibid.*, 1954, **87**, 1865 (cf. C. Grundmann and A. Kreutzberger, *J. Amer. Chem. Soc.*, 1955, **77**, 44).
[76] Y. Asahina and S. Shibata, "Chemistry of Lichen Substances," Japanese Society for the Promotion of Science, Tokyo, 1954.
[77] E. M. Philbin, W. I. A. O'Sullivan, and T. S. Wheeler, *J.*, 1954, 4174.
[78] J. E. Gowan, P. M. Hayden, and T. S. Wheeler, *J.*, 1955, 862.
[79] D. M. Fitzgerald, J. F. O'Sullivan, E. M. Philbin, and T. S. Wheeler, *J.*, 1955, 860.
[80] W. E. Fitzmaurice, W. I. O'Sullivan, E. M. Philbin, and T. S. Wheeler, *Chem. and Ind.*, 1955, 652.

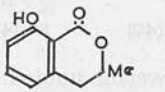
the mechanism of this reaction has now been studied.[81] Flavonoids frequently rearrange in acid media [*e.g.*, (46) ⟶ (47)], probably by ether-scission of the pyrone ring followed by cyclisation in the alternative way.[82]



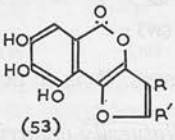
(50)



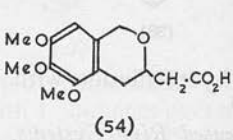
(51)



(52)



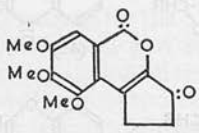
(53)



(54)

Infrared absorption properties of flavones and flavanones have been reported,[83] and reactivity sequences in the methylation of flavone-hydroxy groups and in the demethylation of methyl ethers established.[84] A new type of flavonoid pigment, represented by distemonanthin (50) has been discovered.[85] Details of the synthesis of *leuco*anthocyanidins have been published,[86] and the conformational structure (51) has been proposed for catechin; *epi*catechin is the 3-epimer.[87]

The structure of mellein (ochracin) (52) has been confirmed [88] by synthesis



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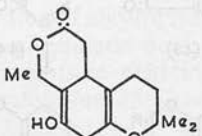

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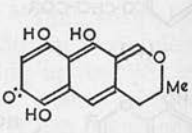

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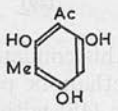

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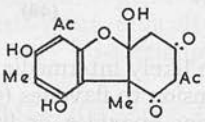
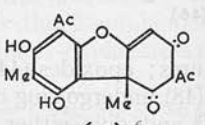


(59)

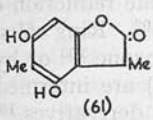


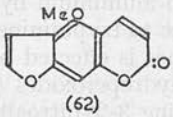
(60)

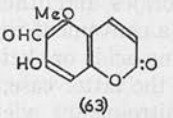
of the (±)-methyl ether, and that of *iso*galloflavin [89] (53; R = CO_2H, R′ = H; or *vice versa*) by degradation to the acid (54). Two groups of workers [90] have synthesised trimethylbrevifolin (55).

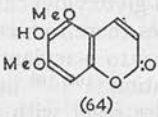
[81] T. A. Geissman and A. Armen, *J. Amer. Chem. Soc.*, 1955, **77**, 1623.
[82] S. K. Mukerjee and T. R. Seshadri, *Chem. and Ind.*, 1955, 271.
[83] B. L. Shaw and T. H. Simpson, *J.*, 1955, 655.
[84] T. H. Simpson and J. L. Beton, *J.*, 1954, 4065.
[85] F. E. King, T. J. King, and P. J. Stokes, *J.*, 1955, 4594.
[86] F. E. King and J. W. Clark-Lewis, *J.*, 1955, 3384.
[87] F. E. King, J. W. Clark-Lewis, and W. F. Forbes, *J.*, 1955, 2948; J. W. Clark-Lewis, *Chem. and Ind.*, 1955, 1218; cf. E. A. H. Roberts, *ibid.*, pp. 631, 1551.
[88] J. Blair and G. T. Newbold, *J.*, 1955, 2871.
[89] J. Grimshaw, R. D. Haworth, and H. K. Pindred, *J.*, 1955, 833.
[90] R. D. Haworth and J. Grimshaw, *Chem. and Ind.*, 1955, 199; K. Bernauer and O. T. Schmidt, *Annalen*, 1955, **591**, 153.

Structure (56) is proposed for fuscin, and the total synthesis of dihydrofuscin methyl ether reported.[91] Purpurogenone (probably 57) has been isolated [92] from a strain of *Penicillium purpurogenum* Stoll.


(61)

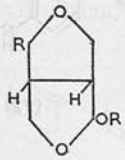

(62)

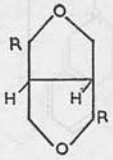

(63)

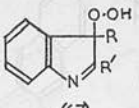

(64)

The total synthesis of usnic acid (60) has been accomplished elegantly; ferricyanide oxidation of methylphloracetophenone (58) affords the hydroxydiketone (59), which is dehydrated by concentrated sulphuric acid to (±)-usnic acid;[93a] this had been resolved previously.[93b] The mode of dimerisation of *p*-cresol derivatives involved in this synthesis appears to be fairly general, and is possibly significant in the biogenesis of dibenzofuran lichen substances.[93a] The formation of dibenzofurans and diquinones from quinones has been discussed.[93c] An important advance is the synthesis of the product (61) of ozonolysis of usnic acid.[94]

Chromic acid oxidation of bergapten (62) gives *apo*xanthoxyletin (63); methylation, and treatment with acid hydrogen peroxide, then gives fraxinol (64). Similar reactions are employed in the conversion of visnagin into baicalein.[95]


(65)


(66)


(67)

(R = 3 : 4-methylenedioxyphenyl)

The structure of sesamolin (65), which with sesamin (66) is mainly responsible for the pyrethrum-synergistic activity of sesame oil, has been confirmed by degradation.[96] Fagarol is identical with (±)-sesamin.[97]

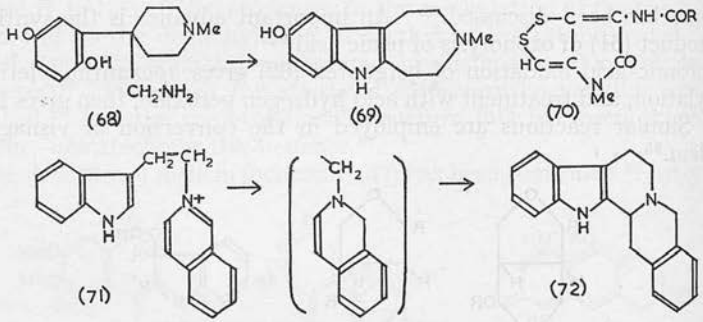
Condensed Ring Systems containing Nitrogen.—Ultraviolet-light absorption by mono- and di-cyclic *N*-heteroaromatic systems has been discussed.[98]

[91] D. H. R. Barton and J. B. Hendrickson, *Chem. and Ind.*, 1955, 682; A. J. Birch, *ibid.*, p. 682.
[92] J. C. Roberts and C. W. H. Warren, *J.*, 1955, 2992.
[93] (*a*) D. H. R. Barton, A. M. Deflorin, and O. E. Edwards, *Chem. and Ind.*, 1955, 1039; (*b*) F. M. Dean, P. Halewood, S. Mongkolsuk, A. Robertson, and W. B. Whalley, *J.*, 1953, 1250; (*c*) F. M. Dean, A. M. Osman, and A. Robertson, *J.*, 1955, 11.
[94] F. M. Dean and A. Robertson, *J.*, 1955, 2166.
[95] A. Schönberg, N. Badran, and N. A. Starkowsky, *J. Amer. Chem. Soc.*, 1955, 77, 5390.
[96] M. Beroza, *ibid.*, p. 332; E. Haslam and R. D. Haworth, *J.*, 1955, 827; H. Erdtman and Z. Pelchowicz, *Chem. and Ind.*, 1955, 567; B. Carnmalm, H. Erdtman, and Z. Pelchowicz, *Acta Chem. Scand.*, 1955, **9**, 1111.
[97] B. Carnmalm and H. Erdtman, *Chem. and Ind.*, 1955, 570.
[98] S. F. Mason, *Chem. Soc. Special Publ.* No. 3, 1955, p. 139.

Monographs have been published dealing with indoles and carbazoles [99] and with condensed thiophen ring systems.[100]

1-Acylated products are obtained from indolylmagnesium bromide and certain lactones.[101] Indoles and oxalyl chloride readily give highly crystalline 3-glyoxyloyl chlorides, and lithium aluminium hydride reduction of the amides therefrom is a convenient route to tryptamines.[102] Ring scission of indoles to *o*-acylamino-acids or -ketones is effected by ozone [103] or by autoxidation; [103, 104] in the latter case, hydroperoxides (67) are intermediates. Indoles react with nitro-olefins, yielding 3-2′-nitroalkyl derivatives,[105] and with α-acetamidoacrylic acid in acetic acid–anhydride, indole gives acetyltryptophan.[106] Piperidine-ring expansion occurs during the ferricyanide oxidation of the substituted derivatives (68), and the *cyclo*heptenoindole (69) is formed.[107]

Ultraviolet-light absorptions and base strengths for quinolines have been reported.[108] The chemistry of Reissert compounds has received further attention,[109] and this subject has been reviewed.[110] Nitration of quinoline



affords only a small yield of nitro-compound, mainly the 3-isomer.[111] 1:2-Dihydro*iso*quinoline has been isolated for the first time; [112] reactive dihydro-*iso*quinolines are intermediates in several interesting syntheses.[113, 114] Thus, reduction [114] of the *N*-substituted *iso*quinolinium salt (71) (from

[99] W. C. Sumper and F. M. Miller, "Chemistry of Heterocyclic Compounds Vol. VIII. Heterocyclic Compounds with Indole and Carbazole Systems," Interscience Publ. Inc., New York, 1954.

[100] H. D. Hartough and S. L. Meisel, "Compounds with Condensed Thiophene Rings," Interscience Publ. Inc., New York, 1954.

[101] A. R. Katritzky and Sir R. Robinson, *J.*, 1955, 2481.

[102] M. E. Speeter and W. C. Anthony, *J. Amer. Chem. Soc.*, 1954, **76**, 6209.

[103] G. Clerc-Bory, M. Clerc-Bory, H. Pacheco, and C. Mentzer, *Bull. Soc. chim. France*, 1955, 1229.

[104] R. J. S. Beer, T. Donavanik, and A. Robertson, *J.*, 1954, 4139.

[105] W. E. Noland, G. M. Christensen, G. L. Sauer, and G. G. S. Dutton, *J. Amer. Chem. Soc.*, 1955, **77**, 456.

[106] H. R. Snyder and J. A. MacDonald, *ibid.*, p. 1257.

[107] J. Harley-Mason and A. H. Jackson, *J.*, 1955, 374.

[108] S. B. Knight, R. H. Wallick, and C. Balch, *J. Amer. Chem. Soc.*, 1955, **77**, 257.

[109] R. F. Collins, *ibid.*, p. 4921; R. L. Cobb and W. E. McEwen, *ibid.*, p. 5042.

[110] W. E. McEwen and R. L. Cobb, *Chem. Rev.*, 1955, **55**, 511.

[111] M. J. S. Dewar and P. M. Maitlis, *Chem. and Ind.*, 1955, 685.

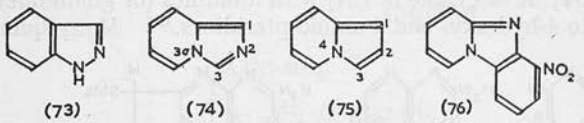
[112] L. M. Jackman and D. I. Packham, *ibid.*, p. 360.

[113] A. R. Battersby, R. Binks, and P. S. Uzzell, *ibid.*, p. 1039.

[114] K. T. Potts and Sir R. Robinson, *J.*, 1955, 2675.

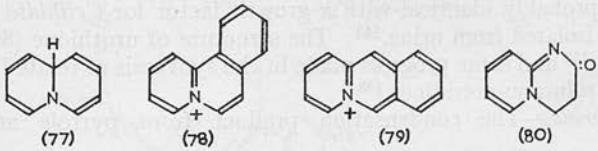
homophthalaldehyde and tryptamine) by lithium aluminium hydride, gives the yohimbine skeleton (72). Bromine forms *N*-bromoacridinium bromides with acridine in carbon tetrachloride, and 3-bromo- and 3 : 7-dibromo-compounds in acetic acid.[115] Thiolutin and aureothricin are yellow crystalline antibiotics isolated from various *Streptomyces*; they have been assigned [116] the unique pyrrolo-1 : 2-dithiole structures (70; R = Me and Et respectively).

A large number of derivatives of indazole (73), including chlorination, nitration, and sulphonation products, has been described.[117] Phosphorus oxychloride cyclises 2-acylaminomethylpyridines to 2 : 3*a*-diazaindenes (74), which very rapidly undergo Friedel–Crafts reactions at $C_{(1)}$, or at $C_{(3)}$ if the former position is substituted.[118] The related pyrrocoline system (75) readily undergoes Friedel–Crafts and *C*-alkylation reactions; [119] in this case,

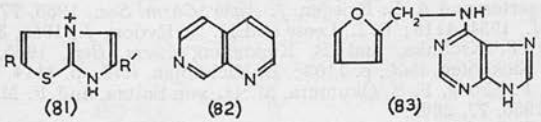


(73) (74) (75) (76)

substitution occurs most easily at $C_{(3)}$. Several derivatives of the system (76) have been made from 2-aminopyridines and 2-chloro-1 : 3-dinitrobenzene.[120] The chemistry of quinolizine (pyridocoline) (77) has been reviewed.[121] Dehydrobenzoquinolizinium salts (78) have been made [122] by treating α-halogenated ketone–2-phenylpyridine quaternary salts with concentrated hydrobromic acid; and acridizinium salts (79) have been



(77) (78) (79) (80)



(81) (82) (83)

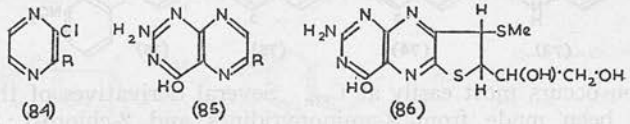
prepared [123] analogously, from 2-formylpyridine and benzyl bromides. 2-Aminopyridine gives the bicyclic system (80) with β-propiolactone, and 2-aminothiazoles behave similarly.[124] Glyoxalinothiazolium salts (81) have been made by several routes,[125] and dihydroglyoxalinothiazolium salts have

[115] R. M. Acheson, T. G. Hoult, and K. A. Barnard, *J.*, 1954, 4142.
[116] W. D. Celmer and I. A. Solomons, *J. Amer. Chem. Soc.*, 1955, **77**, 2861.
[117] R. R. Davies, *J.*, 1955, 2412.
[118] J. D. Bower and G. R. Ramage, *J.*, 1955, 2834.
[119] D. O. Holland and J. H. C. Nayler, *J.*, 1955, 1504.
[120] K. H. Saunders, *J.*, 1955, 3275.
[121] B. S. Thyagarajan, *Chem. Rev.*, 1954, **54**, 1019.
[122] C. K. Bradsher and L. E. Beavers, *J. Amer. Chem. Soc.*, 1955, **77**, 453.
[123] *Idem, ibid.*, p. 4812.
[124] C. D. Hurd and S. Hayao, *ibid.*, p. 117.
[125] A. Lawson and H. V. Morley, *J.*, 1955, 1695; B. Kickhöfen and F. Kröhnke, *Chem. Ber.*, 1955, **88**, 1109.

been made from α-halogenated ketones and tetrahydro-2-thioglyoxaline.[126] Derivatives of the little known 1 : 7-naphthyridine system (82) have been made;[127] polyaza-naphthalene and -indene derivatives are of interest as isosteres of biologically important purines and pyrimidines.[128] A number of glyoxalino-pyridines and -quinolines have been described.[129]

Kinetin (83) has been synthesised from furfurylamine and 6-methylthiopurine;[130] it is a cell-division factor, with the properties of a plant "wound hormone," and has been isolated in crystalline form from autoclaved deoxyribonucleic acid.[131]

Pteridines.—The proceedings of a symposium on pteridines have been published,[132] and infrared and ultraviolet absorptions for a large number of monosubstituted pteridines recorded.[133] Ring scission of hydroxypteridines by acids and alkalis has been studied carefully.[134] Reaction of the chloropyrazines (84; R = CO_2Me or CN) with amidines (or guanidine) provides a new route to 4-hydroxy- and 4-amino-pteridines.[135] Many quinoxalines,[136]



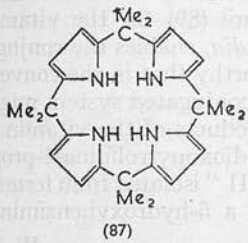
(84) (85) (86)

pteridines,[137] and other polycyclic compounds[138] have been made by the condensation of appropriate *o*-aminonitroso-compounds with cyanoacetic esters. Among new pterins isolated from *Drosophilia* species are [85; R = $CH(OH)\cdot CH(OH)\cdot CH_3$][139] and [85; R = $CH(OH)\cdot CO_2H$].[140] The former is probably identical with a growth factor for *Crithidia fasciculata*, biopterin, isolated from urine.[141] The structure of urothione (86) has been elucidated,[142] and some progress made in the synthesis of related thiophano- and dihydrofurano-pteridines.[143]

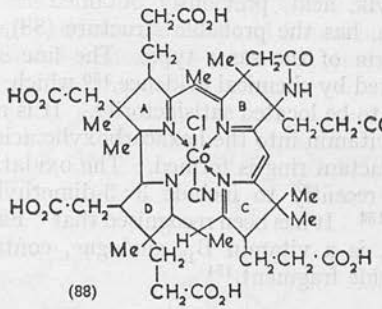
Porphyrins.—The condensation product from pyrrole and acetone

[126] W. Wilson and R. Woodger, *J.*, 1955, 2943.
[127] H. E. Baumgarten and A. L. Krieger, *J. Amer. Chem. Soc.*, 1955, **77**, 2438.
[128] F. L. Rose, *J.*, 1954, 4116; C. L. Leese and H. N. Rydon, *J.*, 1955, 303.
[129] K. Schilling, F. Kröhnke, and B. Kickhöfen, *Chem. Ber.*, 1955, **88**, 1093; F. Kröhnke and B. Kickhöfen, *ibid.*, p. 1103; B. Kickhöfen, *ibid.*, p. 1114.
[130] C. O. Miller, F. Skoog, F. S. Okumura, M. H. von Saltza, and F. M. Strong, *J. Amer. Chem. Soc.*, 1955, **77**, 2662.
[131] C. O. Miller, F. Skoog, M. H. von Saltza, and F. M. Strong, *ibid.*, p. 1392.
[132] G. E. W. Wolstenholme and M. P. Cameron (Editors), Ciba Foundation Symposium on Chemistry and Biology of Pteridines, J. and A. Churchill, London, 1954.
[133] S. F. Mason, *J.*, 1955, 2336.
[134] A. Albert, *J.*, 1955, 2690.
[135] G. P. G. Dick and H. C. S. Wood, *J.*, 1955, 1379; E. C. Taylor, jun., and W. W. Paudler, *Chem. and Ind.*, 1955, 1061.
[136] T. S. Osdene and G. M. Timmis, *J.*, 1955, 2027.
[137] *Idem, ibid.*, p. 2036.
[138] F. C. Copp and G. M. Timmis, *J.*, 1955, 2021; T. S. Osdene and G. M. Timmis, *J.*, 1955, 2032, 2214.
[139] H. S. Forrest and H. K. Mitchell, *J. Amer. Chem. Soc.*, 1955, **77**, 4865.
[140] M. Viscontini, E. Loeser, P. Karrer, and E. Hadorn, *Helv. Chim. Acta*, 1955, **38**, 397, 1222.
[141] E. L. Patterson, H. P. Broquist, A. M. Albrecht, M. H. von Saltza, and E. L. R. Stokstad, *J. Amer. Chem. Soc.*, 1955, **77**, 3167.
[142] R. Tschesche, F. Korte, and G. Heuschkel, *Chem. Ber.*, 1955, **88**, 1251.
[143] R. Tschesche, H. Barkemeyer, and G. Heuschkel, *ibid.*, p. 1258; R. Tschesche and H. Barkemeyer, *ibid.*, p. 976.

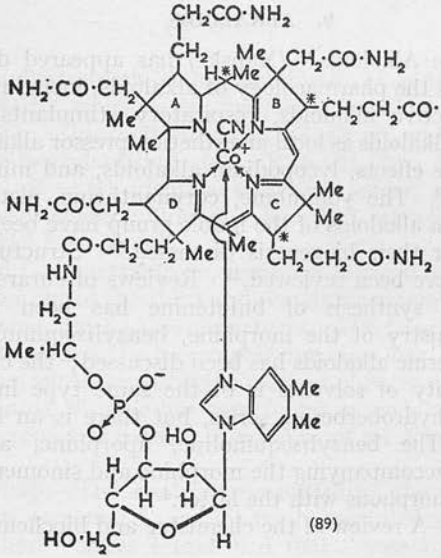
probably [144] has the structure (87), and analogous structures are suggested for the anhydrotetramers obtained from furan and methyl ketones.[145] Factors involved in the stability of metal-porphyrins have been studied



(87)



(88)



(89)

carefully; copper complexes are markedly stabilised, relatively to magnesium complexes, by the introduction of ethoxycarbonyl groups into the pyrrole residues.[146] The structure of chlorophyll [147] and the hydrogenation of porphyrins [148] have been discussed; and a series of papers deals with synthetic tetra-azaporphyrins and intermediate imidines.[149]

[144] P. Rothemund and C. L. Gage, *J. Amer. Chem. Soc.*, 1955, **77**, 3340.
[145] R. G. Ackman, W. H. Brown, and G. F. Wright, *J. Org. Chem.*, 1955, **20**, 1147.
[146] W. S. Caughey and A. H. Corwin, *J. Amer. Chem. Soc.*, 1955, **77**, 1509; A. H. Corwin and M. H. Melville, *ibid.*, p. 2755.
[147] R. P. Linstead, U. Eisner, G. E. Ficken, and R. B. Johns, *Chem. Soc. Special Publ.* No. 3, 1955, p. 83.
[148] M. Whalley, *ibid.*, p. 98.
[149] M. E. Baguley, H. France, R. P. Linstead, and M. Whalley, *J.*, 1955, 3521; G. E. Ficken and R. P. Linstead, *J.*, 1955, 3525; R. P. Linstead and M. Whalley, *J.*, 1955, 3530; J. A. Elvidge and R. P. Linstead, *J.*, 1955, 3536.

The proposal[150, 151] of a detailed structure (89) for vitamin B_{12} is a remarkable achievement, and a full account of this work is eagerly awaited. It was shown in a brilliant *X*-ray crystallographic study[151] that the hexacarboxylic acid, previously obtained[152] by alkaline degradation of the vitamin, has the probable structure (88), and that the vitamin itself is a porphyrin of the same type. The fine structure (89) for the vitamin is supported by chemical evidence,[150] which, *inter alia*, enables the conjugated system to be located satisfactorily. It is noteworthy that in the conversion of the vitamin into the hexacarboxylic acid the conjugated system migrates and a lactam ring is formed. The oxidation products of the vitamin were shown recently to include 3 : 3-dimethyl-2 : 5-dioxopyrrolidine-4-propionamide.[153] It has been recognised that " Factor III " isolated from fermented sewage, is a vitamin B_{12} analogue, containing a 5-hydroxybenziminazole nucleotide fragment.[154]

W. W.

9. ALKALOIDS.

VOLUME V of " The Alkaloids " (Manske) has appeared during the year reviewed. It covers the pharmacology of alkaloids, including narcotics and analgesics, cardio-active alkaloids, respiratory stimulants, antimalarials, uterine stimulants, alkaloids as local anæsthetics, pressor alkaloids, mydriatic alkaloids, curare-like effects, lycopodium alkaloids, and minor alkaloids of unknown structure.[1] The yohimbine, corynantheine, alstonine, cinchonamine, and *Erythrina* alkaloids of the indole group have been reviewed, and possible schemes for their biogenesis discussed.[1a] Structural relations in the alkaloid field have been reviewed.[1b] Reviews of curare alkaloids have appeared.[2] A new synthesis of bufotenine has been reported.[3] The absolute stereochemistry of the morphine, benzyl*iso*quinoline, aporphine, and tetrahydroberberine alkaloids has been discussed; the change of optical rotation with polarity of solvents is of the same type in the benzylisoquinoline and tetrahydroberberine series, but there is an inversion in the aporphine series. The benzyl*iso*quinoline, aporphine, and tetrahydroberberine alkaloids accompanying the morphine and sinomenine alkaloids in Nature are enantiomorphous with the latter.[4]

Tropane Group.—A review of the chemistry and biochemistry of tropane

[150] D. C. Hodgkin, Sir A. R. Todd, and A. W. Johnson, *Chem. Soc. Special Publ.* No. 3, 1955, p. 109; R. Bonnet, J. R. Cannon, A. W. Johnson, I. Sutherland, Sir A. R. Todd, and E. L. Smith, *Nature*, 1955, **176**, 325.

[151] D. C. Hodgkin, J. Pickworth, J. H. Robertson, K. N. Trueblood, R. J. Prosen, and J. G. White, *ibid.*, p. 325.

[152] J. R. Cannon, A. W. Johnson, and Sir A. R. Todd, *ibid.*, 1954, **174**, 1168.

[153] F. A. Kuehl, C. H. Shunk, M. Moore, and K. Folkers, *J. Amer. Chem. Soc.*, 1955, **77**, 4418.

[154] F. M. Robinson, I. M. Miller, J. F. McPherson, and K. Folkers, *ibid.*, p. 5192.

[1] " The Alkaloids," ed. R. H. F. Manske, Academic Press, New York, 1955, Vol. 5.

[1a] V. Boekelheide and V. Prelog, in " Progress in Organic Chemistry," ed. J. W. Cook, Butterworths Scientific Publications, London, 1955, Vol. 3, Chapter 5, p. 218.

[1b] Sir R. Robinson, " The Structural Relations of Natural Products," Oxford Univ. Press, 1955.

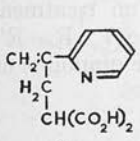
[2] D. Vovet, *Boll. sci. Fac. Chim. ind. Bologna*, 1954, **12**, 172; P. Karrer, *Naturwiss.*, 1955, **176**, 277; P. Karrer and H. Schmid, *Angew. Chem.*, 1955, **67**, 361.

[3] A. Stoll, F. Troxler, J. Peyer, and A. Hofmann, *Helv. Chim. Acta*, 1955, **38**, 1452.

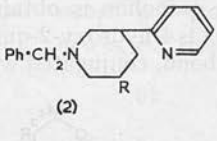
[4] K. W. Bentley and H. M. E. Cardwell, *J.*, 1955, 3252; see also refs. 84 and 85

alkaloids has appeared,[5] and further investigations on their stereochemistry have been reported.[6]

Lupinane Group.—The stereochemistry of the lupin alkaloids has been discussed further,[7] and the synthesis of (±)-cytisine announced. 2-2′-Pyridylallylmalonic acid (1) condensed with benzylamine and formaldehyde, to give 1-benzyl-5-2′-pyridylpiperidine-3-carboxylic acid (2; R = CO_2H).



(1)



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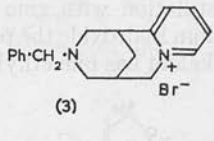

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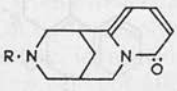

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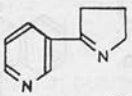


(5)

The derived ethyl ester with lithium aluminium hydride afforded the alcohol (2; R = CH_2·OH), which with hydrogen bromide gave the derivative (2; R = CH_2Br). This quaternised to the salt (3), which on mild oxidation yielded (±)-*N*-benzylcytisine (4; R = CH_2Ph). Debenzylation gave (±)-cytisine (4; R = H), identical with the racemic natural base. This is also a synthesis of caulophylline (4; R = Me) and rhombifoline (4; R = CH_2:CH·CH_2·CH_2).[8]

The alkaloid (−)-spartalupine, found in *Lupinus sericeus* Pursh, is one of the enantiomorphs of the third and remaining racemic pair stereoisomeric with (±)-sparteine and (±)-α-*iso*sparteine. The base has been epimerised to (+)-sparteine and to (+)-α-*iso*sparteine, and has been compared with (±)-spartalupine, synthesised by the method of Šorm and Keil.[9, 10]

(+)-*epi*Lupinine *N*-oxide has been found in seeds of *Lupinus varius* L.; this is the first reported natural occurrence of an *N*-oxide in the lupinane group.[11]

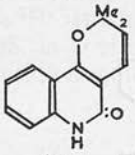
Pyridine Group.—The controversy regarding the occurrence of pelletierine in *Punica granatum* L. cannot yet be regarded as settled.[12] Wibaut and his co-workers [13] have established that "base C," isolated from the plant, is not identical with *iso*pelletierine. It is possible that the original

[5] A. Stoll and E. Jucker, *Chimia* (*Switz.*), 1955, **9**, 25.
[6] A. Heusner, *Z. Naturforsch.*, 1954, **9**b, 683; G. Fodor, J. Tóth, J. Lestyan, and I. W. Vincze, *Szerves Kém. Konf. Debrecen*, 1953, 293; G. Fodor, *Acta Chim. Acad. Sci. Hung.*, 1955, **5**, 379; *Experientia*, 1955, **11**, 129; G. Fodor, J. Tóth, and I. Vincze, *J.*, 1955, 3504; cf. *Ann. Reports*, 1954, **51**, 253.
[7] F. Galinovsky and H. Nesvadba, *Monatsh.*, 1954, **85**, 1300; F. Galinovsky, P. Knoth, and W. Fischer, *ibid.*, 1955, **86**, 1014; J. Ratusky, R. Reiser, and F. Šorm, *Chem. Listy*, 1954, **48**, 1794; *Coll. Czech. Chem. Comm.*, 1955, **20**, 798; cf. *Ann. Reports*, 1954, **51**, 254.
[8] E. E. van Tamelen and J. S. Baran, *J. Amer. Chem. Soc.*, 1955, **77**, 4944.
[9] M. Carmack, B. Douglas, E. W. Martin, and H. Suss, *ibid.*, p. 4435.
[10] F. Šorm and B. Keil, *Coll. Czech. Chem. Comm.*, 1948, **13**, 544.
[11] W. D. Crow and N. V. Riggs, *Austral. J. Chem.*, 1955, **8**, 136.
[12] *Ann. Reports*, 1954, **51**, 254.
[13] J. P. Wibaut, H. C. Beyerman, U. Hollstein, Y. M. F. Muller, and E. Greuell, *Proc. k. ned. Akad. Wetenschap.*, 1955, **58**, *B*, 56.

" pelletierine " of Hess and Eichel [14] is identical with an unidentified base found in the bark.

The tobacco alkaloid myosmine is best represented as 2-3′-pyridyl-Δ^1-pyrroline (5). It shows a strong infrared band characteristic of a >C=N- group conjugated with an aromatic ring, but no band in the >NH region.[15]

Quinoline Group.—The pyranoquinoline alkaloid flindersine has been proved to have the angular structure (6) by a lengthy series of degradations. On distillation with zinc dust quinoline is obtained; on treatment with potassium hydroxide the product is 4-hydroxy-2-quinolone (7; R = R′ = H). The alkaloid has one ethylenic bond, conjugated with the quinoline nucleus.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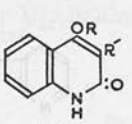
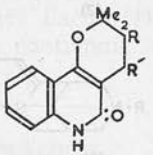

(7)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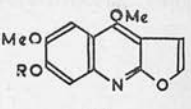

(9)

Oxidation gives flindersinic acid (7; R = $CMe_2 \cdot CO_2H$; R′ = CO_2H), which is further degraded by hydrolysis to carbon dioxide, α-hydroxy*iso*butyric acid, and the quinolone (7; R = R′ = H). Flindersinic acid, when boiled with 95% ethanol, yields the acid (7; R = H, R′ = CO_2H) and the corresponding ethyl ester, and the constitution of the latter has been proved by synthesis. Similar degradations have been carried out on chlorodeoxyflindersine and *N*-methylflindersine. The analogous aldehydic degradation products, α-hydroxy*iso*butyraldehyde and the compound (7; R = H, R′ = CHO) are obtained by oxidation of flindersine with osmium tetroxide to the glycol (8; R = R′ = OH), followed by periodate oxidation and hydrolysis.[16] Final confirmation of the structure (6) is provided by synthesis.[17] Reaction of 4-hydroxy-2-quinolone (7; R = R′ = H) with β-methylcrotonyl chloride gives the ester (7; R = $Me_2C{:}CH \cdot CO$, R′ = H) which undergoes a Fries rearrangement and cyclisation to the pyranoquinolone (8; R = H, R′ = :O). Reduction of the ketonic carbonyl group yields the alcohol (8; R = H, R′ = OH), which on dehydration affords flindersine (6).

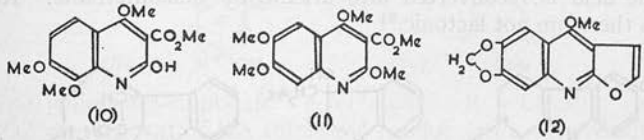
Dictamnic acid has been proved to have structure (7; R = Me, R′ = CO_2H), by unambiguous synthesis; dictamnine therefore has a linear structure.[16]

2 : 3 : 4-Trimethoxy-10-methylacridone has been found in the bark of *Evodia alata* F. Muell.; it was identified by synthesis.[18] Evolatine, from the same source, is a furanoquinoline alkaloid isomeric with evoxine.[19] It has been degraded by methods similar to those used for evoxine; on potash fusion it gives the phenolic base (9; R = H), which on methylation affords kokusaginine (9; R = Me), and on treatment with acid it is converted

[14] K. Hess and A. Eichel, *Ber.*, 1917, **50**, 1192.
[15] B. Witkop, *J. Amer. Chem. Soc.*, 1954, **76**, 5597.
[16] R. F. C. Brown, J. J. Hobbs, G. K. Hughes, and E. Ritchie, *Austral. J. Chem.*, 1954, **7**, 348.
[17] R. F. C. Brown. G. K. Hughes, and E. Ritchie, *Chem. and Ind.*, 1955, 1385.
[18] R. J. Gell, G. K. Hughes, and E. Ritchie, *Austral. J. Chem.*, 1955, **8**, 114.
[19] *Ann. Reports*, 1954, **51**, 255.

into the ketone (9; R = $Me_2CH{\cdot}CO{\cdot}CH_2$), isomeric with evoxoidine. Evolatine is therefore [9; R = $Me_2C(OH){\cdot}CH(OH){\cdot}CH_2$].[18]

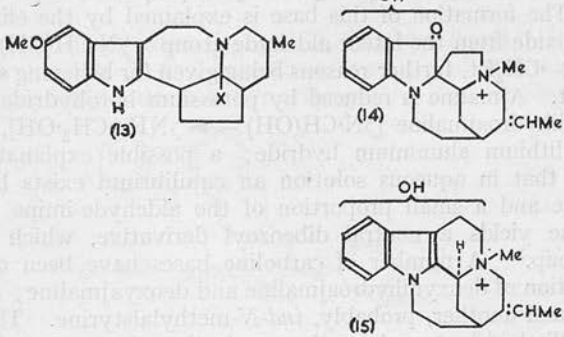
Methyl skimmianinate has been proved by synthesis to have structure (10); this provides confirmatory evidence for the linear structure of skimmianine. Similarly, methyl *O*-methylkokusagininate (11) has been synthesised, proving that kokusaginine has a linear, tricyclic structure.[20]



The alkaloid maculine, from *Flindersia maculosa* Lindl., is 6 : 7-methylenedioxydictamnine (12). On hydrogenolysis it yields 3-ethyl-2 : 4-dihydroxy-6 : 7-methylenedioxyquinoline, identified by synthesis.[21]

Indole Group.—A review of ergot alkaloids has appeared.[22] The stereochemistry of lysergic and dihydrolysergic acid [23] and their amides [24] has been discussed. A series of lumi-compounds has been obtained from ergot alkaloids and other lysergic acid derivatives by ultraviolet irradiation in aqueous acid solution; the 9 : 10-double bond in the lysergic acid residue is hydrated by this procedure.[25]

The stereochemistry of the *allo*yohimbanes,[26] yohimbane, corynantheidane, and related compounds [27] has been discussed. The alkaloid voacan-

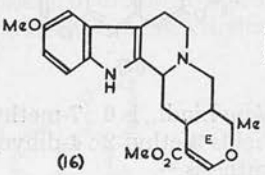


gine, $C_{22}H_{28}O_3N_2$, from *Voacanga thouarsii*, gives on hydrolysis methanol and an acid which is easily decarboxylated to ibogaine; it is therefore a methoxycarbonylibogaine, of probable structure (13; X = CO_2Me).[28]

[20] R. F. C. Brown, *Austral. J. Chem.*, 1955, **8**, 121.
[21] R. J. Gell, G. K. Hughes, and E. Ritchie, *ibid.*, p. 422.
[22] M. Semonsky, *Cesk. Farm.*, 1955, **4**, 198.
[23] A. Stoll, T. Petrzilka, J. Rutschmann, A. Hofmann, and H. H. Günthard, *Helv. Chim. Acta*, 1954, **37**, 2039.
[24] A. Stoll and A. Hofmann, *ibid.*, 1955, **38**, 421.
[25] A. Stoll and W. Schlientz, *ibid.*, p. 585.
[26] E. Wenkert and L. H. Liu, *Experientia*, 1955, **11**, 302.
[27] M.-M. Janot, R. Goutarel, A. Le Hir, G. Tsatsas, and V. Prelog, *Helv. Chim. Acta*, 1955, **38**, 1073.
[28] M.-M. Janot and R. Goutarel, *Compt. rend.*, 1955, **241**, 986.

Karrer and his associates have described extensive investigations on indole alkaloids of curare.[29, 30] Of these, fluorocurine and mavacurine have been assigned partial structures (14) and (15), on degradative and spectroscopic evidence.[30]

The structure (16) is now preferred for aricine. On alkaline hydrolysis aricinic acid is obtained, which differs in molecular formula from aricine by CH_2; the acid is reconverted into aricine by diazomethane. Ring E in aricine is therefore not lactonic.[31]



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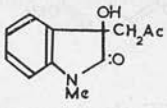

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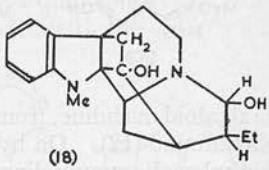


(18)

Interest in alkaloids of *Rauwolfia* spp. continues to increase.[32] A welcome attempt to clarify ambiguities in nomenclature amongst alkaloids of *R. serpentina* Benth. has been made, the intense activity in this field having resulted in the assignment of several names to single compounds.[33]

New structures have been proposed for ajmaline.[32] The formation of 3-acetonyl-3-hydroxy-1-methyloxindole (17) on oxidation confirms the presence of a dihydro-*N*-methylindole system in the alkaloid. When heated with nickel, both ajmaline and *iso*ajmaline yield decarbonoajmaline, $C_{19}H_{26}ON_2$, a secondary base giving *n*-butyric, propionic, and acetic acid on oxidation. The formation of this base is explained by the elimination of carbon monoxide from the latent aldehyde group : $\rangle N{\cdot}CH(OH){\cdot}CHEt \longrightarrow \rangle NH + CO + {\cdot}CH_2Et$, further reasons being given for believing such a group to be present. Ajmaline is reduced by potassium borohydride in aqueous solution to dihydroajmaline [$\rangle N{\cdot}CH(OH) \longrightarrow \rangle NH \cdot CH_2{\cdot}OH$], but is not reduced by lithium aluminium hydride; a possible explanation of this difference is that in aqueous solution an equilibrium exists between the carbinolamine and a small proportion of the aldehyde-imine forms. Dihydroajmaline yields a neutral dibenzoyl derivative, which has a free hydroxyl group.[34] A number of carboline bases have been obtained by dehydrogenation of deoxydihydroajmaline and deoxyajmaline; one of these is alstyrine, and another, probably, *ind*-*N*-methylalstyrine. The structure (18) for the alkaloid best explains these and other observations.[34, 35] Other investigators prefer a cyclic semiacetal structure (19), there being some doubt about the position of the methyl group and the form of ring E.[36]

[29] H. Asmis, E. Bächli, E. Giesbricht, J. Kebrle, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, 1954, **37**, 1968; E. Giesbricht, H. Meyer, E. Bächli, H. Schmid, and P. Karrer, *ibid.*, p. 1974; H. Asmis, H. Schmid, and P. Karrer, *ibid.*, p. 1983; H. Asmis, E. Bächli, H. Schmid, and P. Karrer, *ibid.*, p. 1993.

[30] H. Bickel, H. Schmid, and P. Karrer, *ibid.*, 1955, **38**, 649.

[31] A. Stoll, A. Hofmann, and R. Brunner, *ibid.*, p. 270; cf. *Ann. Reports*, 1954, **51**, 258.

[32] *Ibid.*, p. 256.

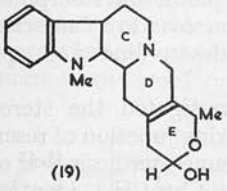
[33] D. D. Phillips and M. S. Chadha, *Chem. and Ind.*, 1955, 414.

[34] Sir R. Robinson and co-workers, *ibid.*, p. 285.

[35] F. C. Finch, J. D. Hobson, Sir R. Robinson, and E. Schlittler, *ibid.*, p. 653.

[36] A. Chatterjee and S. Bose, *Sci. and Cult.*, 1955, **20**, 606.

Confirmation of the location of the three vicinal substituents in ring E of reserpic acid (20; R = CO_2H, R′ = OH) has been provided by degrad-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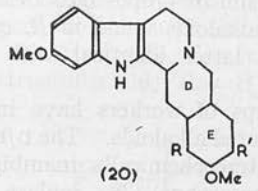
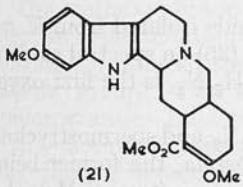

(20)

ation. The toluene-*p*-sulphonic ester (20; R = CO_2Me, R′ = *p*-Me·C_6H_4·SO_3) on removal of the sulphonyl residue yields methyl anhydroreserpate (21), which is the enol ether of a β-keto-ester, since it is readily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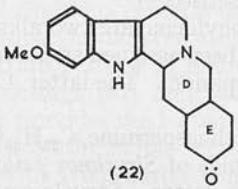
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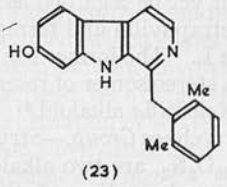
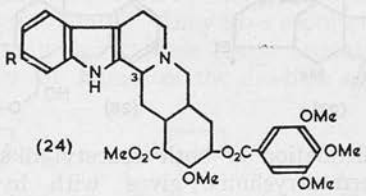

(23)

hydrolysed and decarboxylated to the ketone reserpone (22). The same toluenesulphonate, on reduction with lithium aluminium hydride, affords reserpinol (20; R = CH_2·OH, R′ = H), which on dehydrogenation yields 7-hydroxy-yobyrine (23); the structure of the last is proved by synthesis of its methyl ether.[37]

A new alkaloid, deserpidine, $C_{32}H_{38}O_8N_2$, has been found in *R. canescens*. On hydrolysis it yields 3 : 4 : 5-trimethoxybenzoic acid and methyl deserpidate. It is probably a demethoxyreserpine (24; R = H), since its ultra-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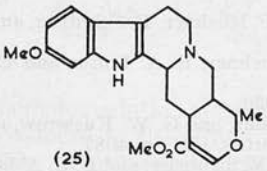
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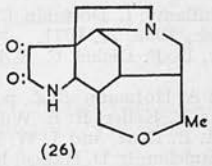

(26)

violet absorption curve is almost coincident with that of yohimbine 3 : 4 : 5-trimethoxybenzoate.[38] Methyl deserpidate has been converted into α-yohimbine by a simple series of reactions, an inversion occurring at $C_{(3)}$.

[37] C. F. Huebner, H. B. MacPhillamy, A. F. St. André, and E. Schlittler, *J. Amer. Chem. Soc.*, 1955, **77**, 472.

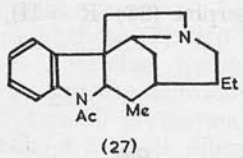
[38] E. Schlittler, P. R. Ulshafer, M. L. Pandow, R. M. Hunt, and L. Dorfman, *Experientia*, 1955, **11**, 64.

Hence deserpidine, and also reserpine, are derivatives of 3-*epi*-α-yohimbine,[39] which has itself been found in *R. serpentina*.[40] Structures (24; R = H) and (24; R = OMe) are proposed for deserpidine and reserpine respectively.[39] Amongst other alkaloids found in *R. canescens* are canescine [41, 42] and recanescine,[43] the latter identical with deserpidine and, probably, with canescine.

Several groups of workers have investigated the stereochemistry of reserpine and related alkaloids. The D/E ring junction of reserpine is proved to be *cis* by the stereochemically unambiguous synthesis [44, 45] of 11-methoxy-*allo*yohimbane (reserpane) (22; replace CO by CH_2), identical, apart from the racemic character of the synthetic material, with the Wolff–Kishner reduction product of reserpone (22). Considerable divergence of view is found regarding the absolute stereochemistry of reserpine, and the matter cannot yet be regarded as settled.[45, 46]

Tetraphyllin and tetraphyllicine are two alkaloids isolated from *R. tetraphylla* L. The former has been assigned structure (25) on spectral evidence: it is a stereoisomer of reserpinine. The latter, $C_{20}H_{26}N_2$, is the first oxygen-free *Rauwolfia* alkaloid.[47]

Strychnos *Group*.—Strychnospermine, $C_{22}H_{28}O_3N_2$, and spermostrychnine, $C_{21}H_{26}O_2N_2$, are two alkaloids of *Strychnos psilosperma*, the former being a methoxy-derivative of the latter. Strychnospermine (two *C*-Me) shows ultraviolet absorption characteristic of a 1-acetyl-2 : 3-dihydroindole, and deacetylstrychnospermine of a 1 : 2-dihydroindole, both with a methoxy-group in the benzene ring; the infrared absorption confirms this. Of the two nitrogen atoms, one [N(*a*)] is weakly and the other strongly basic; the methoxyl group is *meta* to N(*a*). The oxidation of demethylstrychnospermine or spermostrychnine with chromic acid yields *apo*spermostrychnine



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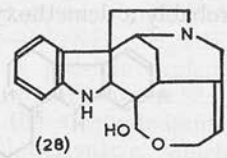
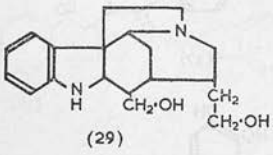


(28)

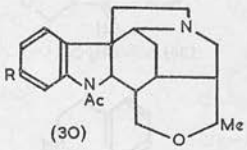
(26), and zinc dust distillation of both deacetyl-alkaloids gives 3-ethyl-pyridine. Deacetylspermostrychnine gives with hydrogen bromide

[39] H. B. MacPhillamy, L. Dorfman, C. F. Huebner, E. Schlittler, and A. F. St. André, *J. Amer. Chem. Soc.*, 1955, **77**, 1071.
[40] F. E. Bader, D. F. Dickel, C. F. Huebner, R. A. Lucas, and E. Schlittler, *ibid.*, p. 3547.
[41] A. Stoll and A. Hofmann, *ibid.*, p. 820.
[42] M. W. Klohs, F. Keller, R. E. Williams, and G. W. Kusserow, *ibid.*, p. 4084.
[43] N. Neuss, H. E. Boaz, and J. W. Forbes, *ibid.*, p. 4087.
[44] E. E. van Tamelen, P. D. Hance, K. V. Siebrasse, and P. E. Aldrich, *ibid.*, p. 38[illegible].
[45] C. F. Huebner, *Chem. and Ind.*, 1955, 1186.
[46] P. A. Diassi, F. L. Weisenborn, C. M. Dylion, and O. Wintersteiner, *J. Amer. Chem. Soc.*, 1955, **77**, 2028, 4687; C. F. Huebner and E. Wenkert, *ibid.*, p. 4180; E. van Tamelen and P. D. Hance, *ibid.*, p. 4692; M.-M. Janot, R. Goutarel, A. Le [illegible] G. Tsatsas, and V. Prelog, *Helv. Chim. Acta*, 1955, **38**, 1073; H. B. MacPhillamy, C. Huebner, E. Schlittler, A. F. St. André, and P. R. Ulshafer, *J. Amer. Chem. Soc.*, 19[illegible] **77**, 4335; C. F. Huebner, H. B. MacPhillamy, E. Schlittler, and A. F. St André, *Experientia*, 1955, **11**, 303.
[47] C. Djerassi and J. Fishman, *Chem. and Ind.*, 1955, 627.

bromo-derivative, which is reduced by zinc dust to deoxydihydrospermostrychnine (27). This base has been synthesised from the Wieland–Gumlich aldehyde (28), reduction of which in two stages gives the glycol (29), whence successive treatment with hydrogen bromide and zinc dust–acetic acid, followed by acetylation, yields the base (27). Spermostrychnine and strychnospermine have therefore structures (30; R = H and OMe respectively).[48]

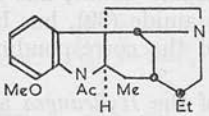


(29)

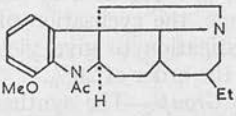


(30)

The methoxyl group of aspidospermine is at the 7-position, as in vomicine; on demethylation *N*-acetylaspidosine is obtained, which shows no OH band in the infrared spectrum because of hydrogen bonding involving the phenolic group. Two structures (31) and (32) have been suggested for aspidospermine; the latter provides the better explanation of the formation of 3 : 5-diethylpyridine and 3-ethylindole on zinc dust distillation, but is more difficult to reconcile with Woodward's biogenetic scheme.[49]

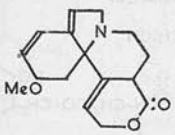


(31)

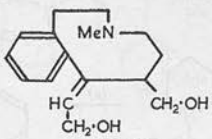


(32)

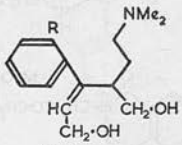
Erythrina *Group*.—The structure (33) has been proposed for α-erythroidine,[50] which has been degraded by methods similar to those employed for the isomeric β-erythroidine.[51] Dihydro-α-erythroidinol on Hofmann degradation gives the aromatic base (34). Oxidation of this base yields phthalic acid; it is an isomer of the des-base of dihydro-β-erythroidine.



(33)



(34)



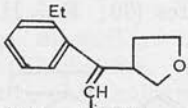
(35)

Further Hofmann degradation gives the vinyl derivative (35; R = $CH{:}CH_2$), which can be reduced to the ethyl derivative (35; R = Et); oxidation of the latter yields *o*-ethylbenzoic acid. A final stage of Hofmann decomposition affords the tetrahydrofuran (36), which on mild oxidation is converted into the ketone (37), and on more vigorous oxidation into *o*-ethylbenzoic acid. These and other observations show that α-erythroidine has

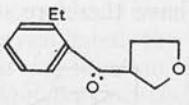
[48] F. A. L. Anet and Sir R. Robinson, *J.*, 1955, 2253.
[49] B. Witkop and J. B. Patrick, *J. Amer. Chem. Soc.*, 1954, **76**, 5603.
[50] J. C. Godfrey, D. S. Tarbell, and V. Boekelheide, *ibid.*, 1955, **77**, 3342.
[51] *Ann. Reports*, 1952, **49**, 225.

the same carbon skeleton as β-erythroidine, but the two differ in the arrangement of the lactone ring.[50]

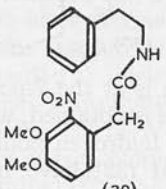
The structure (38) proposed for *apo*erysopine [52] has been confirmed by synthesis of its dimethyl ether.[53]



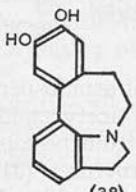
(36)



(37)



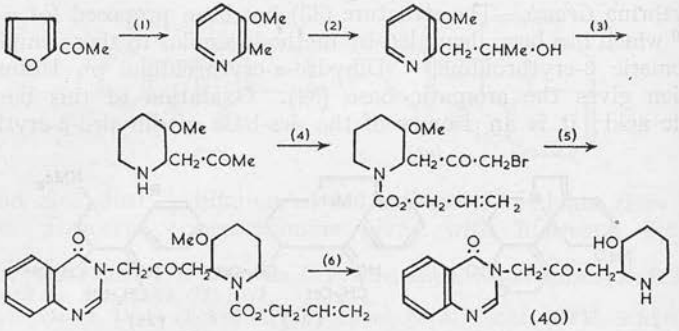
(39)



(38)

Aporphine Group.—Späth and Hromatka's synthesis [54] of *apo*morphine dimethyl ether, hitherto held in considerable doubt, has been vindicated. The critical stage, the cyclisation of the amide (39), has been found [55] on careful re-investigation to give yields of the corresponding 3 : 4-dihydro-*iso*quinoline of the order of 20%.

Quinazolone Group.—The synthesis of the *Hydrangea* alkaloid (40) has been described,[56] the route being as shown in outline.



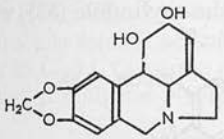
Reagents: (1) *a*, Aq. NH_3; *b*, methyln. (2) Me·CHO. (3) *a*, $3H_2$; *b*, CrO_3. (4) *a*, Br_2–HBr; *b*, $Cl·CO_2·CH_2·CH:CH_2$. (5) Quinazol-4-one. (6) Aq. HCl.

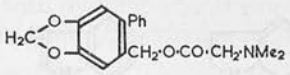
Phenanthridine Group.—Further evidence in support of the structure (41) for lycorine has been advanced. On periodate oxidation, dihydro-

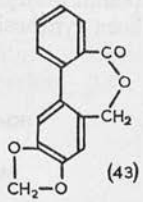
[52] M. Carmack, B. C. McKusick, and V. Prelog, *Helv. Chim. Acta*, 1951, **34**, 1601.
[53] K. Wiesner, Z. Valenta, A. J. Manson, and F. W. Stonner, *J. Amer. Chem. Soc.*, 1955, **77**, 675.
[54] E. Späth and O. Hromatka, *Ber.*, 1929, **62**, 325.
[55] D. H. Hey and A. L. Palluel, *Chem. and Ind.*, 1955, 40.
[56] B. R. Baker and F. J. McEvoy, *J. Org. Chem.*, 1955, **20**, 136.

lycorinone yields a 4-acyl*iso*carbostyril derivative, the formation of which can be explained satisfactorily if lycorine is a disecondary glycol.[57]

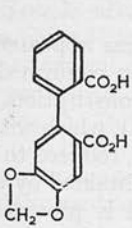
The structure of tazettine methine has been proved to be (42) by synthesis.[58] Reduction of tazettine with lithium aluminium hydride yields

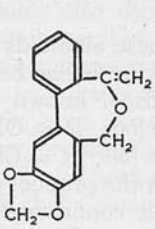

(41)

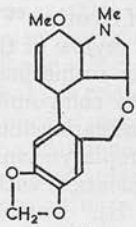

(42)

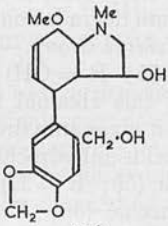

(43)

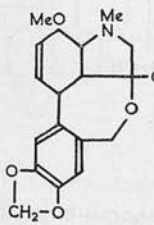
*seco*tazettine, which on dehydration gives anhydro*seco*tazettine. Hofmann degradation (two stages) of the latter affords a nitrogen-free product which does not show an infrared carbonyl band but forms a 2 : 4-dinitrophenylhydrazone. On oxidation, first the lactone (43) and then the diphenic acid (44) are obtained, both structures being proved by synthesis. The *pseudo*carbonyl Hofmann product therefore has structure (45), anhydro*seco*-

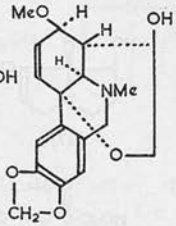

(44)

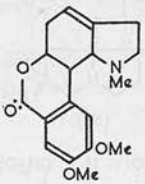

(45)

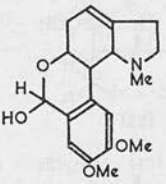

(46)


(47)


(48)


(49)


(50)

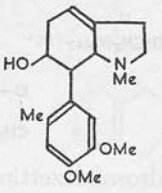

(51)

tazettine (46), *seco*tazettine (47), and tazettine (48).[59] The same structure has been proposed for tazettine on different grounds [60] and is preferred to the earlier structure (49) proposed on the basis of extensive degradations.[61]

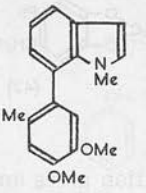
Infrared measurements suggest that homolycorine, $C_{18}H_{21}O_4N$, an

[57] S. Takagi, W. I. Taylor, S. Uyeo, and H. Yajima, *J.*, 1955, 4003; cf. *Ann. Reports*, 1954, **51**, 261.
[58] W. I. Taylor, S. Uyeo, and H. Yajima, *J.*, 1955, 2962.
[59] T. Ikeda, W. I. Taylor, and S. Uyeo, *Chem. and Ind.*, 1955, 1088.
[60] R. J. Highet and W. C. Wildman, *ibid.*, p. 1159.
[61] E. Wenkert, *Experientia*, 1954, **10**, 476.

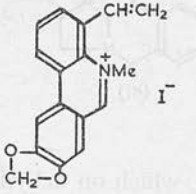
alkaloid of *Lycoris radiata* Herb., is a δ-lactone [62] and not a γ-lactone. Lycorenine, $C_{18}H_{23}O_4N$, from the same source, gives homolycorine on mild oxidation; it is a cyclic semiacetal. Structures (50) and (51) have been proposed for homolycorine and lycorenine respectively,[62] and have been confirmed by Wolff–Kishner reduction of lycorenine to the dihydrodeoxy-compound (52). This on dehydrogenation affords the arylindole (53), which has been synthesised from the Emde base of lycorine.[64]



(52)



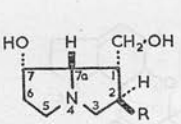
(53)



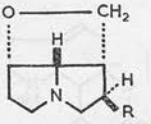
(54)

The product formed by reaction of lycorine anhydromethine with methyl iodide is the phenanthridinium salt (54); previous workers may have obtained anhydrolycorine methiodide, which has the same m. p. as this salt since anhydrolycorine is known to accompany lycorine anhydromethine in the Hofmann degradation of lycorine.[65]

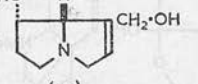
Pyrrolizidine Group.—A review of these alkaloids has appeared.[66] The structure (55; R = OH) for rosmarinecine [67] has been confirmed by conversion of this alkaloid into compounds of known constitution. On dehydration it gives anhydrorosmarinecine (56; R = OH), which with thionyl chloride yields anhydrochloroplatynecine (56; R = Cl), reduced to anhydroplatynecine (56; R = H), identical with the product obtained by dehydrating platynecine (55; R = H). Further confirmation is provided by the synthesis of rosmarinecine from retronecine (57), which with perbenzoic acid



(55)



(56)



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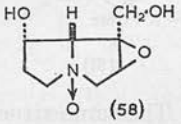
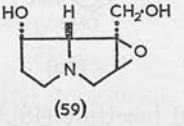


(58)



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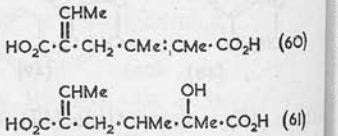
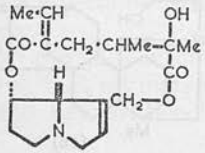


yields epoxyisatinecine (58). Reduction then affords epoxyretronecine (59), further reduced to rosmarinecine (55; R = OH). The various transformations indicate the similarity in stereoconfiguration of the hydro-

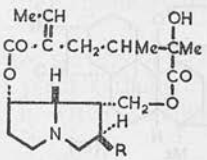
[62] T. Kitagawa, W. I. Taylor, S. Uyeo, and H. Yajima, *J.*, 1955, 1066.
[63] H.-G. Boit, L. Paul, and W. Stender, *Chem. Ber.*, 1955, **88**, 133.
[64] S. Uyeo and H. Yajima, *J.*, 1955, 3392; *Ann. Reports*, 1954, **51**, 261.
[65] T. Shingu, S. Uyeo, and H. Yajima, *J.*, 1955, 3557.
[66] F. L. Warren, *Fortschr. Chem. org. Naturstoffe*, 1955, **12**, 198.
[67] M. F. Richardson and F. L. Warren, *J.*, 1943, 452.

groups in rosmarinecine and platynecine, where the 7-hydroxyl group is *cis* to the 1-hydroxymethyl group, and both are *trans* to the 7*a*-hydrogen atom.[68]

The alkaloid rosmarinine on dehydration gives anhydrorosmarinine, hydrolysed to rosmarinecine (55; R = OH) and, probably, anhydrosenecic acid (60). The toluene-*p*-sulphonic ester of the same alkaloid yields on hydrolysis senecic acid (61) and *epi*rosmarinecine (55; R = OH, and inversion at $C_{(2)}$); the same ester with pyridine affords senecionine, with elimination of toluene-*p*-sulphonic acid. Senecionine is therefore (62), and



(62)

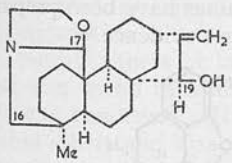


(63)

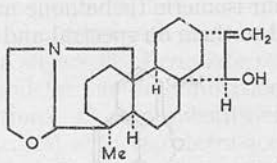
rosmarinine and platyphylline have structures (63; R = OH and H respectively).[69] Integerrimine, known to be the *trans*-form of senecionine,[70] therefore has structure (62; Me·CH replaced by HC·Me).[69]

The *Senecio* alkaloids also occur as their *N*-oxides.[69] Hieracifoline [71] and pterophine [72] have been shown by paper chromatography each to be mixtures of senecionine and seneciphylline.[73]

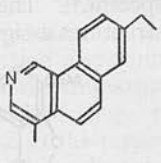
Diterpene Group.—Interest in alkaloids related to the tricyclic diterpenes is increasing.[74] On the basis of oxidation and dehydrogenation, structures (64) and (65) have been proposed for veatchine and garryine respectively.[75] Selenium dehydrogenation of both alkaloids gives the benzo*iso*quinoline (66), identified by synthesis.[75, 76] Cuauchichicine, a new member of the group,



(64)



(65)

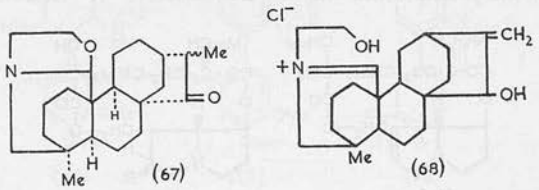


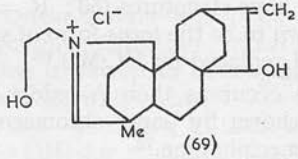
(66)

found in *Garrya laurifolia* Hartw., is isomeric with veatchine, but contains no $C{:}CH_2$ group and is ketonic. On degradation it yields products identical with, or similar to, those obtained from veatchine under the same conditions, and its p*K* value is comparable with that of veatchine, rather than of

[68] L. J. Dry, M. J. Koekemoer, and F. L. Warren, *J.*, 1955, 59.
[69] M. J. Koekemoer and F. L. Warren, *ibid.*, p. 63.
[70] M. Kropman and F. L. Warren, *J.*, 1950, 700.
[71] R. H. F. Manske, *Canad. J. Res.*, 1939, **17**, *B*, 8.
[72] H. L. de Waal, *Nature*, 1940, **146**, 777.
[73] C. C. J. Culvenor and L. W. Smith, *Chem. and Ind.*, 1954, 1386; R. Adams and M. Gianturco, *J. Amer. Chem. Soc.*, 1956, **78**, 398.
[74] For a review, see E. S. Stern, in " The Alkaloids," Manske and Holmes, Academic Press, New York, 1954, Vol. 4, p. 275.
[75] K. Wiesner, R. Armstrong, M. F. Bartlett, and J. A. Edwards, *J. Amer. Chem. Soc.*, 1954, **76**, 6068.
[76] M. F. Bartlett and K. Wiesner, *Chem. and Ind.*, 1954, 542.

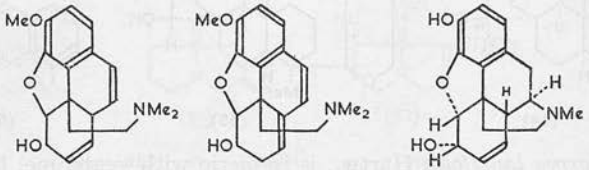
garryine, which suggests that the oxazolidine ring is fused to $C_{(17)}$ rather than to $C_{(16)}$. Structure (67) explains these and other observations most satisfactorily.[77, 78] Laurifoline,* an isomeric alkaloid from the same source, is readily isomerised by acids to cuauchichicine and by hot ethanol to isolaurifoline; it is 19-*epi*veatchine (64; with inversion at $C_{(19)}$).[78] The infrared absorption spectra of atisine and *iso*atisine hydrochlorides show a band characteristic of the $\rangle C{:}\overset{+}{N}\langle$ group, and in the ultraviolet region the salts show more intense absorption above 220 mμ than the bases. This can





be explained if the salts are quaternary chlorides of structures (68) and (69) respectively.[79] Some observations have been made on the stereochemistry of diterpenoid alkaloids, in relation to basic strength.[80]

Morphine Group.—Structures (70) and (71) for α- and β-codeimethine have been confirmed by considerations of their mode of formation, reactions and spectra.[81] The four isomeric thebainone methines have been prepared and structures assigned to them on spectral and other evidence.[82]



X-Ray crystallographic determinations support the view that (–)-morphine has the stereochemistry (72) or its mirror image,[83] and a co

[77] C. Djerassi, C. R. Smith, S. K. Figdor, J. Herran, and J. Romo, *J. Amer. Chem. Soc.*, 1954, **76**, 5889.
[78] C. Djerassi, C. R. Smith, A. E. Lippman, S. K. Figdor, and J. Herran, *ibid.*, 1955, **77**, 4801.
[78a] *Idem, ibid.*, p. 6633.
[79] S. W. Pelletier and W. A. Jacobs, *Chem. and Ind.*, 1955, 1385.
[80] K. Wiesner and J. A. Edwards, *Experientia*, 1955, **11**, 255.
[81] K. W. Bentley and A. F. Thomas, *J.*, 1955, 3237.
[82] K. W. Bentley and H. M. E. Cardwell, *ibid.*, p. 3245.
[83] M. Mackay and D. C. Hodgkin, *ibid.*, p. 3261.
* Laurifoline has been re-named garryfoline.[78a]

sideration of molecular-rotation differences establishes that the latter formula represents the absolute stereochemical structure of the natural alkaloid.[4] Similar conclusions have been reached from a study of the degradation of thebaine [84] and of *N*-nor*apo*codeine.[85]

A. R. P.

10. CARBOHYDRATES.

Three years have elapsed since the last Report on polysaccharides.[1] The greater part of the Section is therefore devoted to this subject, and discussion of other aspects of carbohydrate chemistry has been confined to a few selected topics.

A recent book [2] provides authoritative surveys of methods for the isolation, identification, and estimation of plant carbohydrates. Attention is also drawn to the new edition [3] of E. Ott's "Cellulose and Cellulose Derivatives."

Monosaccharides and oligosaccharides.

General Methods.—The high efficiency of a mixture of methyl iodide and silver oxide in dimethylformamide as a methylating agent has been demonstrated with sucrose,[4] D-galactal,[5] and α-solanine,[6] where a single treatment gave products which no longer showed the characteristic infrared absorption of the hydroxyl group.

A preliminary communication [7] draws attention to the value of the alkali-labile 2 : 4-dinitrophenyl residue for protecting the amino-group in glucosamine reactions. Formation of an acid-labile orthoformate has been used to protect the reducing group of a digalacturonic acid before reduction with lithium aluminium hydride.[8] Disaccharides can be smoothly reduced with sodium borohydride;[9] an earlier observation that this reduction is accompanied by fission of the glycosidic link has not been confirmed.

Degradation of 3-*O*-methyl-aldoses with periodate provides a useful route to 2-methyl ethers of lower sugars.[10] Periodate oxidation also forms the basis of a useful micromethod for the determination of the ring structures of sugar residues.[11] Reactions of monosubstituted aldoses (*e.g.*, monomethyl ethers and disaccharides) with lead tetra-acetate in the presence of potassium acetate follow definite oxidation patterns depending on the position of substitution; this enables structural determinations to be

[84] J. Kalvoda, P. Buchschacher, and O. Jeger, *Helv. Chim. Acta*, 1955, **38**, 1847.
[85] H. Corrodi and E. Hardegger, *ibid.*, p. 2038.
[1] E. J. Bourne, *Ann. Reports*, 1952, **49**, 235.
[2] "Modern Methods of Plant Analysis," Ed. K. Paech and M. V. Tracey, Springer Verlag, Berlin, 1955, Vol. II.
[3] "Cellulose and Cellulose Derivatives," Ed. E. Ott and H. M. Spurlin, Interscience Publ., New York, 1954 and 1955.
[4] R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, 1955, **67**, 32.
[5] R. Kuhn and H. H. Baer, *Chem. Ber.*, 1955, **88**, 1537.
[6] R. Kuhn, I. Löw, and H. Trischmann, *ibid.*, p. 1492.
[7] P. F. Lloyd and M. Stacey, *Chem. and Ind.*, 1955, 917.
[8] J. K. N. Jones and W. W. Reid, *J.*, 1955, 1890.
[9] W. J. Whelan and K. Morgan, *Chem. and Ind.*, 1955, 1449.
[10] G. W. Huffman, B. A. Lewis, F. Smith, and D. R. Spriestersbach, *J. Amer. Chem. Soc.*, 1955, **77**, 4346.
[11] M. Viscontini, D. Hoch, and P. Karrer, *Helv. Chim. Acta*, 1955, **38**, 642; see also F. Smith and J. W. van Cleve, *J. Amer. Chem. Soc.*, 1955, **77**, 3091.

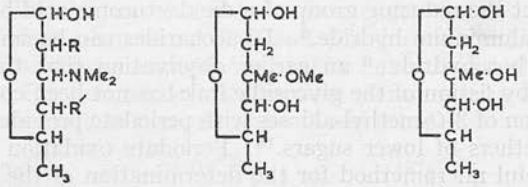
carried out on a few milligrams of material.[12] As in periodate oxidation, the aldoses appear to be oxidised as cyclic hemiacetals yielding formates. The preparative value of oxidation with lead tetra-acetate is illustrated by the production of formates of D-erythrose and L-glyceraldehyde from D-glucose and L-arabinose, respectively.[13]

Paper chromatography of carbohydrates has been reviewed.[14] Chromatography on paper impregnated with boric acid facilitated the separation of certain methyl ethers and polyols,[15] and the value of borate complexes in the preparative separation of glycosides and sugars has been emphasized.[16] The ionophoretic behaviour of many glucose derivatives in alkaline borate buffer has been related to their structure.[17]

The classical method of separating 4-substituted monosaccharides from mixtures, by conversion of the other components into methyl furanosides with methanolic hydrogen chloride at room temperature, has been applied successfully to oligosaccharides.[18] It is noteworthy that 2 : 3 : 6-tri-*O*-methyl-D-mannose does not form a furanoside under the usual mild conditions; this can be used to separate it from 2 : 3 : 6-tri-*O*-methyl-D-glucose.[19]

The molecular weights of osazones of mono- and oligo-saccharides can conveniently be determined by a spectrophotometric method.[20, 21]

Sugars from Antibiotics.—The amino-sugars, mycaminose (1; R = R′ = OH; from magnamycin)[22] and rhodosamine (probably 1; R = H, R′ = OH; from rhodomycin),[23] are closely related to desosamine (1; R = OH, R′ = H; from erythromycin), which was discussed in last year's Report.[24] The stereochemistry of these compounds remains to be



(1) (2) (3)

elucidated. Determination of the structure of cladinose (2),[25] another hydrolysis product of erythromycin, shows that it is related to mycarose (3),[26] the branched-chain sugar from magnamycin. The resemblance be

[12] A. S. Perlin, *Analyt. Chem.*, 1955, **27**, 396.
[13] A. S. Perlin and C. Brice, *Canad. J. Chem.*, 1955, **33**, 1216.
[14] G. N. Kowkabany, *Adv. Carbohydrate Chem.*, 1954, **9**, 303; F. A. Isherwood, *Brit. Med. Bull.*, 1954, **10**, No. 3, 202; D. J. Bell, ref. 2, p. 1.
[15] G. R. Barker and D. C. C. Smith, *Chem. and Ind.*, 1954, 19.
[16] M. V. Lock and G. N. Richards, *J.*, 1955, 3024.
[17] A. B. Foster and M. Stacey, *J.*, 1955, 1778.
[18] S. A. Barker, E. J. Bourne, and D. M. O'Mant, *Chem. and Ind.*, 1955, 425.
[19] P. A. Rebers and F. Smith, *J. Amer. Chem. Soc.*, 1954, **76**, 6097.
[20] V. C. Barry, J. E. McCormick, and P. W. D. Mitchell, *J.*, 1955, 222.
[21] R. Kuhn, A. Gauhe, and H. H. Baer, *Chem. Ber.*, 1954, **87**, 289.
[22] F. A. Hochstein and P. P. Regna, *J. Amer. Chem. Soc.*, 1955, **77**, 3353.
[23] H. Brockmann and E. Spohler, *Naturwiss.*, 1955, **42**, 154.
[24] J. C. P. Schwarz, *Ann. Reports*, 1954, **51**, 262.
[25] P. F. Wiley and O. Weaver, *J. Amer. Chem. Soc.*, 1955, **77**, 3422.
[26] P. P. Regna, F. A. Hochstein, R. L. Wagner, jun., and R. B. Woodward, *ibid.*, 1953, **75**, 4625.

tween the amino-sugars and branched-chain sugars derived from magnamycin and erythromycin is of interest in view of their similar "microbiological spectra."

Phenylhydrazine Derivatives.—The formation of a formazan when solutions of phenylhydrazine derivatives are coupled in pyridine with diazotised aniline has been used as a diagnostic test for the group CH:N·NHAr.[27] This reaction indicates that two of the three known modifications of glucose phenylhydrazone have cyclic structures, while the third is acyclic.[27] The behaviour of glucose phenylosazone, which couples in alkaline ethanol (but not in pyridine) to give a violet formazan, has been interpreted in terms of an open-chain structure in which the two phenylhydrazine residues are linked by a hydrogen bond.[28] However, it seems necessary to reconcile this structure with the observation [29] that glucose phenylosazones mutarotate in dry pyridine. The close resemblance between the ultraviolet absorption spectra of the sugar osazones and of glycerosazone provides further evidence for the open-chain structure, although it is rather surprising that these spectra differ markedly from that of methylglyoxal bisphenylhydrazone.[20] *N*-Alkylphenylosazones may differ in structure from the unalkylated compounds; in the former the hydrazine residue at $C_{(2)}$ is more reactive,[30] while in the latter the hydrazine residue at $C_{(1)}$ generally shows the greater reactivity.[29] The mechanism of osazone formation has been discussed.[31] Hydrazine exchange can be involved when two different hydrazines are present.

Inositols.—Seven of the nine possible stereoisomers of inositol were already known; the remaining two, *neo*inositol (123/456) and *cis*-inositol (123456/), have now been synthesised. *neo*Inositol was prepared from (−)-inositol by inversion of the configuration of two adjacent carbon atoms *via* an epoxide intermediate.[32] *cis*-Inositol, which has been separated from the mixture obtained on hydrogenation of hexahydroxybenzene,[33] is of considerable interest, as the chair conformation must involve three axial hydroxyl groups situated on the same side of the ring. A new *C*-methylinositol has been encountered in algæ,[34] and three new *O*-methyl*myo*inositols have been isolated from natural sources; [35] the detailed structures of these compounds remain to be elucidated. The synthesis of DL-bornesitol by methylation of 1 : 3 : 4 : 5 : 6-penta-*O*-acetyl*myo*inositol involves migration of an acetyl residue from an equatorial to an axial hydroxyl group.[36] A number of quercitols (*cyclo*hexanepentols), conduritols (*cyclo*hexenetetrols), and *cyclo*hexanetetrols have been synthesised from *myo*- and *epi*-inositol; [37]

[27] L. Mester and Á. Major, *J. Amer. Chem. Soc.*, 1955, **77**, 4297.
[28] L. Mester, *ibid.*, p. 4301.
[29] F. Weygand, H. Grisebach, K.-D. Kirchner, and M. Haselhorst, *Chem. Ber.*, 1955, **88**, 487.
[30] G. Henseke and H.-J. Binte, *ibid.*, p. 1167.
[31] G. Henseke and H. Dalibor, *ibid.*, p. 521; G. Henseke and M. Bautze, *ibid.*, p. 62; V. C. Barry and P. W. D. Mitchell, *Nature*, 1955, **175**, 220.
[32] S. J. Angyal and N. K. Matheson, *J. Amer. Chem. Soc.*, 1955, **77**, 4343.
[33] S. J. Angyal and D. J. McHugh, *Chem. and Ind.*, 1955, 947.
[34] B. Lindberg and J. McPherson, *Acta Chem. Scand.*, 1954, **8**, 1875; B. Lindberg, *ibid.*, 1955, **9**, 1097; H. Bouveng and B. Lindberg, *ibid.*, p. 168.
[35] V. Plouvier, *Compt. rend.*, 1955, **241**, 765, 983.
[36] L. Anderson and A. M. Landel, *J. Amer. Chem. Soc.*, 1954, **76**, 6130.
[37] G. E. McCasland and E. C. Horswill, *ibid.*, p. 2373; G. E. McCasland and J. M. Reeves, *ibid.*, 1955, **77**, 1812.

one of the tetrols was also obtained from *cyclo*hexa-1 : 4-diene by the action of Prévost's reagent (silver benzoate and iodine).[38]

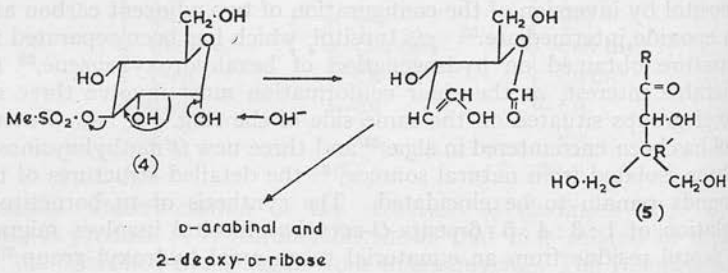
Oligosaccharides.—Investigation of the terpenoid glycoside, stevioside, which is about 300 times as sweet as sucrose, has shown that two of the three glucose units are present as a 1 : 2-linked disaccharide.[39] The remaining glucose unit is independently attached to a hindered carboxyl group of the aglycone by esterification at $C_{(1)}$, and this glucose residue is eliminated as 1 : 6-anhydro-D-glucose by the action of potassium hydroxide. 2 : 3 : 4 : 6-Tetra-*O*-acetyl-1-*O*-(2 : 4 : 6-trimethylbenzoyl)-β-D-glucose also gives 1 : 6-anhydro-D-glucose on treatment with alkali.[40] These unusual reactions presumably involve " alkyl "–oxygen fission.

A branched trisaccharide, *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[3-D-glucopyranosyl-(1→3)]-D-galactose, has been obtained from the alkaloid α-solanine.[6] The related alkaloid, α-chaconine, contains a branched trisaccharide in which two L-rhamnopyranosyl units are linked to the 2- and 4-hydroxyl groups of D-glucose.[41] Human milk continues to yield interesting oligosaccharides, one of which is 2′-*O*-α-L-fucopyranosyl-lactose.[42]

The observation[43] that disaccharide osazones are hydrolysed to monosaccharide osazones under conditions which leave disaccharides unchanged suggests a method for the stepwise degradation of oligosaccharides.

Oligosaccharides encountered during work on polysaccharides are discussed in the latter part of this Report.

Miscellaneous.—The reaction of 2-*O*-sulphonyl derivatives of arabinose, xylose, and fucose with alkali proceeds with inversion at $C_{(2)}$ giving ribose, lyxose, and talomethylose, respectively.[44, 45] This inversion provides a



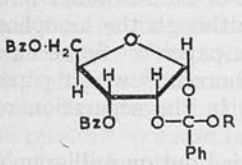
promising route to some otherwise inaccessible sugar derivatives. 3-O-Methanesulphonyl-D-glucose (4) reacts with alkali to give 2-deoxy-D-ribose and D-arabinal, $C_{(1)}$ being eliminated as formate;[45] this interesting reaction has a formal analogy in the alkaline cleavage of 3-tosyl esters of ster-

[38] G. E. McCasland and E. C. Horswill, *J. Amer. Chem. Soc.*, 1954, **76**, 1654.
[39] H. B. Wood, jun., R. Allerton, H. W. Diehl, and H. G. Fletcher, jun., *J. Org. Chem.*, 1955, **20**, 875.
[40] H. B. Wood, jun., and H. G. Fletcher, jun., *J. Amer. Chem. Soc.*, 1956, **78**, 7[illegible]; see also F. Micheel and G. Baum, *Chem. Ber.*, 1955, **88**, 2020.
[41] R. Kuhn, I. Löw, and H. Trischmann, *Chem. Ber.*, 1955, **88**, 1690.
[42] R. Kuhn, H. H. Baer, and A. Gauhe, *ibid.*, p. 1135.
[43] P. A. Finan and P. S. O'Colla, *Chem. and Ind.*, 1955, 1387.
[44] J. K. N. Jones and W. H. Nicholson, *J.*, 1955, 3050.
[45] D. C. C. Smith, *Chem. and Ind.*, 1955, 92.

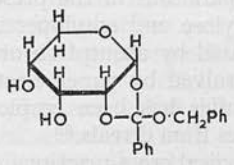
3 : 5-diols. 2-Deoxy-DL-ribose has been synthesised from but-2-yne-1 : 4-diol,[46] and the branched-chain sugars (±)-cordycepose (5; R = R′ = H) and (±)-apiose (5; R = H, R′ = OH) have been built up from bromoacetal and ethyl sodiomalonate.[47] Self-condensation of dihydroxyacetone gives dendroketose (5; R = CH_2·OH, R′ = OH), which can be degraded to apionic acid (5; R = R′ = OH).[48] 4 : 4-Di-*C*-hydroxymethyl-D-threose, an aldose related to dendroketose, and 2-*C*-hydroxymethyl-D-xylose have been prepared from D-fructose by use of the cyanohydrin synthesis.[49]

Recent additional evidence shows the importance of neighbouring-group participation in the reactions of acetohalogeno-sugars and aldose acetates, although the relative reactivities of the α- and β-anomers cannot be entirely ascribed to this effect. This work and other studies on reaction mechanisms in carbohydrate chemistry are discussed in the section on Theoretical Chemistry.

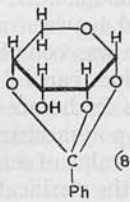
Reactions involving neighbouring-group participation have led to several interesting new orthobenzoic acid derivatives. Hydrolysis of tri-*O*-benzoyl-β-D-ribofuranosyl bromide gave 2 : 3 : 5-tri-*O*-benzoyl-β-D-ribose and the crystalline orthoacid (6; R = H), which rapidly rearranged to 2 : 3 : 5-tri-*O*-benzoyl-β-D-ribose in the presence of a trace of base.[50] The orthoacid



(6)



(7)



(8)

(6; R = H) can also be prepared by hydrogenolysis of the orthoester (6; R = benzyl), which is obtained when the above ribofuranosyl halide reacts with benzyl alcohol in the presence of quinoline. Reaction of tri-*O*-benzoyl-β-D-ribopyranosyl bromide with benzyl alcohol in the presence of quinoline, followed by debenzoylation, yields the labile orthoester (7),[51] and under weakly acidic conditions this readily gives the crystalline 1 : 2 : 4-*O*-orthobenzoyl-α-D-ribopyranose (8); 2 : 3 : 6-*O*-orthobenzoyl-D-fructofuranose has been obtained in a similar way.[52]

Polysaccharides.

The period reviewed has seen the extended application of chromatographic methods in the determination of the detailed structure of polysaccharides; as a result many new polysaccharides have been investigated, and previously unknown structural features have been revealed in substances whose general structures were already known. Progress has been limited in some investigations by difficulties in isolating individual molecular species

[46] M. M. Fraser and R. A. Raphael, *J.*, 1955, 4280.
[47] R. A. Raphael and C. M. Roxburgh, *J.*, 1955, 3405.
[48] L. M. Utkin, *Doklady Akad. Nauk S.S.S.R.*, 1949, **67**, 301.
[49] R. J. Woods and A. C. Neish, *Canad. J. Chem.*, 1954, **32**, 404.
[50] R. K. Ness and H. G. Fletcher, jun., *J. Amer. Chem. Soc.*, 1954, **76**, 1663.
[51] H. G. Fletcher, jun., and R. K. Ness, *ibid.*, 1955, **77**, 5337.
[52] B. Helferich and L. Bottenbruch, *Chem. Ber.*, 1953, **86**, 651; B. Helferich and W. Schulte-Hürmann, *ibid.*, 1954, **87**, 977.

from the complex mixtures present in natural sources. The greatest single need in this field, therefore, is for new and powerful methods for fractionation of polysaccharides, both for separation of structurally distinct molecular species and for resolution of closely-related substances of the same general type.

A chromatographic method for the separation of acid mucopolysaccharides has been described, which makes use of a " carrier " amine to increase the solubility of such polysaccharides in the mobile organic phase;[53] not only was the separation of chondroitin-sulphuric acid and hyaluronic acid effected by this technique, but the resolution of hyaluronic acid fractions of different molecular weight was achieved without apparent degradation. Fractional precipitation with ammonium sulphate has proved of value in the separation of glucosans and araboxylans present in the water-soluble gum and hemicellulose fractions of cereal grains.[54, 55] An electrophoretic method for the analysis of neutral polysaccharides in borate buffer has been reported;[56] separations were effected between amylose and amylopectin, and between yeast mannan and yeast glycogen. Electrophoretic separation of polysaccharides in alkali appears to be limited to the gross separation of charged from neutral molecules, *e.g.*, sodium alginate and laminarin.[57] Paper-ionophoretic separations in the presence of borate buffer have been achieved between amylose and amylopectin, although the ionophoresis of amylose was complicated by absorption on the paper.[58] Some mucopolysaccharides can be resolved by paper electrophoresis,[59] whilst paper ionophoresis in borate buffer has been employed in the separation of some neutral polysaccharides from cereals.[60]

The value of serological cross-reactions, carried out on milligram quantities, in the clarification of structural chemical relationships has been demonstrated in their application to the structure of lung galactan.[61] The immunological specificities of galactose-containing polysaccharides of known general structure have been determined.[62] The nitrogen and sulphur contents of the polymers obtained by the condensation of periodate-oxidised polysaccharides with *iso*nicotinhydrazide and thiosemicarbazide give a measure of the proportion of sugar units attacked by periodate.[63] This method provides a useful check on direct measurements of periodate consumption by polysaccharides, and in addition enables a polysaccharide containing both 1 : 3- and 1 : 4-linkages to be distinguished from a mixture of polysaccharides each containing only one type of linkage. The optical rotations of the carbanilates of polyglucosans in pyridine and morpholine have been shown to be dependent on the position and anomeric type of linkage.[64]

Cellulose and Hemicelluloses.—The use of paper-chromatograph

[53] G. S. Berenson, S. Roseman, and A. Dorfman, *Biochim. Biophys. Acta*, 1955, **17**,
[54] I. A. Preece and K. G. Mackenzie, *J. Inst. Brewing*, 1952, **58**, 353, 457.
[55] I. A. Preece and R. Hobkirk, *ibid.*, 1953, **59**, 385; 1954, **60**, 490.
[56] D. H. Northcote, *Biochem. J.*, 1954, **58**, 353.
[57] J. R. Colvin, W. H. Cook, and G. A. Adams, *Canad. J. Chem.*, 1952, **30**, 603.
[58] A. B. Foster, P. A. Newton-Hearn, and M. Stacey, *J.*, 1956, 30.
[59] K. G. Rienits, *Biochem. J.*, 1953, **53**, 79.
[60] I. A. Preece and R. Hobkirk, *Chem. and Ind.*, 1955, 257.
[61] M. Heidelberger, Z. Dische, W. B. Neely, and M. L. Wolfrom, *J. Amer. Chem. Soc.*, 1955, **77**, 3511.
[62] M. Heidelberger, *ibid.*, p. 4308.
[63] V. C. Barry, J. E. McCormick, and P. W. D. Mitchell, *J.*, 1954, 3692.
[64] I. A. Wolff, P. R. Watson, and C. E. Rist, *J. Amer. Chem. Soc.*, 1953, **75**, 4

techniques together with methylation end-group assay has enabled one terminal group in a thousand to be detected in celluloses, which have been methylated with rigorous exclusion of oxygen to exclude degradation.[65] The values obtained for the chain lengths of methylated celluloses by end-group assay were in reasonable agreement with those obtained from physical measurements; it is necessary, therefore, to abandon the loop structure proposed for cellulose by W. N. Haworth.[66] Kinetic studies of the acid hydrolysis of cellulose [67] have failed to provide evidence for the presence of periodic abnormally-sensitive linkages of the type previously proposed.[68] In the case of cotton cellulose, such acid-sensitive linkages may be present in celluloses regenerated from cuprammonium and cupriethylenediamine solutions.

The close association of cellulose and other cell-wall polysaccharides continues to be emphasised, but there is still no conclusive evidence for the presence or absence of formal linkages between these substances. For example, sugars other than glucose have been detected in the hydrolysates from jute [69] and wheat-straw [70] α-celluloses. Careful fractionation of white-spruce α-cellulose nitrates [71] has yielded fractions of high and of low molecular weight which contain both glucose and mannose residues. The close association of glucose and mannose residues in coniferous woods has also been indicated by the isolation of a disaccharide, composed of glucose and mannose units, from the acetolysis of slash-pine α-cellulose;[72] hemicelluloses composed of glucose and mannose residues occur in such woods, and these results may arise from the incomplete removal of these components.

The cell-wall polysaccharides associated with cellulose are generally referred to as hemicelluloses, although this definition lacks precision in respect of both chemical structure and biological function. In the following account, these polysaccharides are classified according to their basic structural features and are thus differentiated from those polysaccharides whose chemical structures and biological functions are more clearly defined, *e.g.*, the gums exuded from certain plants and reserve polysaccharides such as starch and fructosans. The hemicellulose group of polysaccharides has been reviewed in E. L. Hirst's Pedler Lecture.[73]

Xylans.—A large number of polysaccharides of this group have received attention during the last three years. All the xylans from land plants, so far examined, contain backbones of β-1 : 4-linked D-xylopyranose residues, but they differ in the number and nature of the other sugar residues present; even within a single botanical species, it is clear that several different, but closely related, xylans may be present. The L-arabofuranose residues present in many of these hemicelluloses are integral parts of the xylan molecules, usually occurring as terminal groups and probably linked directly to the main chains as single-unit side-chains; there is no evidence at present for

[65] D. I. McGilvray, *J.*, 1953, 2577.
[66] W. N. Haworth, *Chem. and Ind.*, 1939, 917.
[67] A. Sharples, *J. Polymer Sci.*, 1954, **13**, 393; **14**, 95.
[68] E. V. Schulz, *J. Polymer Sci.*, 1948, **3**, 365; E. Pacsu, *ibid.*, 1947, **2**, 565.
[69] D. B. Das, M. K. Mitra, and J. F. Wareham, *Nature*, 1953, **171**, 613.
[70] G. A. Adams and C. T. Bishop, *ibid.*, p. 28.
[71] T. E. Timell, *Pulp and Paper Mag. Canada*, 1955, **56**, 104.
[72] J. G. Leech, *T.A.P.P.I.*, 1952, **35**, 249.
[73] E. L. Hirst, *J.*, 1955, 2974.

the occurrence, in the hemicellulose group, of arabans similar to those found in the pectic substances. Methylation studies have shown that wheat-straw,[74, 75] esparto,[76] corn-cob,[75] and wheat-leaf [77] xylans contain L-arabofuranose residues linked to the main chain through $C_{(3)}$ of the D-xylose residue, whilst in the more highly branched araboxylans from wheat endosperm,[78] arabinose residues are also linked through $C_{(2)}$ of doubly-branched xylose residues. Additional evidence that the arabinose residues of wheat-straw xylans are integral parts of the molecule has been obtained by the isolation from enzymic hydrolysis of a series of oligosaccharides containing both xylose and arabinose units.[79] Wheat-leaf [77] and some wheat-straw [74, 80, 81] xylans also contain D-glucuronic acid units as a part of their molecular structure. In wheat-straw xylans, some of the main chains of xylose residues are linear,[81] whilst others contain branch points.[82]

Xylans containing D-glucuronic acid (mainly as the 4-methyl ether), but no arabinose, residues are found in elm,[83] beech,[84] and birch [85] woods. Structural investigation of beechwood hemicellulose A [84] has shown that every tenth D-xylopyranose residue carries a single 4-*O*-methyl-D-glucuronic acid residue attached as a side-chain through $C_{(2)}$. The isolation of xylobiose and of 2-*O*-(4-*O*-methyl-D-glucuronosyl)-D-xylose from the partial acid hydrolysis of black spruce and Scots pine shows that xylans of the same general type are also present in coniferous woods.[86] Methylation of flax-straw hemicellulose [87] has indicated a structural similarity to the wood rather than to the cereal-straw hemicelluloses, in that the backbone of xylose residues carries single 4-*O*-methyl-D-glucuronic acid units linked as side-chains, again through $C_{(2)}$ of the xylose residues; 2 : 4-di-*O*-methyl-L-rhamnose was also isolated from the hydrolysis of the methylated polysaccharide, but its structural significance is not yet clear. Xylans of still greater complexity occur in the hemicelluloses of corn cobs (hemicellulose B) [88–90] and wheat bran,[91] where some L-arabinose residues are present in non-terminal positions. Several oligosaccharides have been isolated from corn-cob hemicellulose B on partial acid hydrolysis, and it is clear from the isolation of four aldobiouronic acids [88] that both D-glucuronic acid and 4-*O*-methyl-D-glucuronic acid residues are linked to separate D-xylose residues through $C_{(2)}$ and $C_{(4)}$. The isolation of 2-*O*-α-D-xylopyranosyl-L-arabinose

[74] G. A. Adams, *Canad. J. Chem.*, 1952, **30**, 698; A. Roudier, *Compt. rend.*, 1953, **237**, 840; *Assoc. tech. ind. papetière Bull.*, 1954, 53.
[75] I. Ehrenthal, R. Montgomery, and F. Smith, *J. Amer. Chem. Soc.*, 1954, **76**, 3304.
[76] G. O. Aspinall, E. L. Hirst, R. W. Moody, and E. G. V. Percival, *J.*, 1953, 1677.
[77] G. A. Adams, *Canad. J. Chem.*, 1954, **32**, 186.
[78] R. Montgomery and F. Smith, *J. Amer. Chem. Soc.*, 1955, **77**, 2834, 3325.
[79] C. T. Bishop and D. R. Whitaker, *Chem. and Ind.*, 1955, 119.
[80] C. T. Bishop, *Canad. J. Chem.*, 1953, **31**, 134.
[81] G. O. Aspinall and R. S. Mahomed, *J.*, 1954, 1731.
[82] C. T. Bishop, *Canad. J. Chem.*, 1955, **33**, 1073.
[83] I. Tachi and N. Yamamori, *J. Agric. Chem. Soc. Japan*, 1951—52, **25**, 12, 130, 2
[84] G. O. Aspinall, E. L. Hirst, and R. S. Mahomed, *J.*, 1954, 1734.
[85] J. Saarnio, K. Wathén, and C. Gustafsson, *Acta Chem. Scand.*, 1954, **8**, 825.
[86] A. R. N. Gorrod and J. K. N. Jones, *J.*, 1954, 2522.
[87] F. Smith and J. D. Geerdes, *J. Amer. Chem. Soc.*, 1955, **77**, 3572.
[88] R. L. Whistler and L. Hough, *ibid.*, 1953, **75**, 4918; R. L. Whistler, H. E. Conrad, and L. Hough, *ibid.*, 1954, **76**, 1668.
[89] R. L. Whistler and D. I. McGilvray, *ibid.*, 1955, **77**, 1884.
[90] *Idem, ibid.*, p. 2212.
[91] G. A. Adams, *Canad. J. Chem.*, 1955, **33**, 56.

shows that non-terminal arabinose residues are present in the polysaccharide, but does not indicate whether they are present in the furanose or pyranose form. It is clear from methylation studies that wheat-bran hemicellulose is a highly branched polysaccharide containing D-xylose and L-arabinose, each in three or four states of combination, together with uronic acid residues.[91]

Galactans and Galactoarabans.—The galactan from *Strychnos nux-vomica* seeds [92] has been shown by methylation and periodate oxidation studies to be an essentially linear β-1 : 4-galactan, similar to that previously isolated from *Lupinus albus* pectin.[93] The arabogalactan from Jeffrey pine [94] contains a much higher proportion of L-arabinose residues than the ε-galactan from larch,[95,96] and in the highly branched molecular structure the majority of L-arabinose residues occur in the furanose form as terminal groups; there is no evidence that any of the arabinose residues are present in the pyranose form, as in larch ε-galactan.[96] The backbone of the pine galactan is probably composed of 1 : 6-linked D-galactopyranose units, some of which are branched through $C_{(3)}$. The galactoaraban from Japanese larch,[97] like those from European [95] and Western [98] larches, contains galactose and arabinose residues in the ratio of 6 : 1; fractionation of the methylated polysaccharide failed to yield components having different physical and chemical properties. As a result of the application of Barry's degradation [99] to the galactogen of the snail *Helix pomatia*, a dichotomously branched structure has been advanced to replace the comb-like structure of a backbone of galactose residues with single unit branches previously put forward for this polysaccharide on the basis of methylation results.[100] It is now clear from the results of cross-precipitin reactions [61] that the uronic acid-containing moieties associated with beef-lung galactan [101] arise from a contaminating polysaccharide.

Mannans, Glucomannans, and Galactomannans.—Methylation has shown that the mannose-containing polysaccharides of *Iris ochroleuca* and *I. sibirica* are composed of equal proportions of 1 : 4-linked D-mannose and D-glucose residues together with a small number of D-galactopyranose residues linked solely as non-reducing end-groups.[102] A re-investigation, by chromatographic methods, of the sugars obtained on hydrolysis of the methylated ivory-nut mannans A and B indicates that both polysaccharides contain mixtures of molecular species, terminated by D-mannopyranose and D-galactopyranose residues, respectively; [103] both species are linear, but in addition to the 1 : 4-linked D-mannose units, some mannose units are present in one or both types of molecule linked through $C_{(1)}$ and $C_{(6)}$. It is clear from

[92] P. Andrews, L. Hough, and J. K. N. Jones, *J.*, 1954, 806.
[93] E. L. Hirst, J. K. N. Jones, and W. O. Walder, *J.*, 1947, 1225.
[94] W. H. Wadman, A. B. Anderson, and W. Z. Hassid, *J. Amer. Chem. Soc.*, 1954, **76**, 4097.
[95] W. G. Campbell, E. L. Hirst, and J. K. N. Jones, *J.*, 1948, 774.
[96] J. K. N. Jones, *J.*, 1953, 1692.
[97] I. Tachi and N. Yamamori, *J. Agric. Chem. Soc., Japan*, 1953, **27**, 139.
[98] E. V. White, *J. Amer. Chem. Soc.*, 1941, **63**, 2871; 1942, **64**, 302, 1507, 2838.
[99] P. O'Colla, *Proc. Roy. Irish Acad.*, 1953, **55**, *B*, 165.
[100] E. Baldwin and D. J. Bell, *J.*, 1938, 1461; D. J. Bell and E. Baldwin, *J.*, 1941, 125.
[101] M. L. Wolfrom, G. Sutherland, and M. Schlamowitz, *J. Amer. Chem. Soc.*, 1952, **74**, 4883.
[102] P. Andrews, L. Hough, and J. K. N. Jones, *J.*, 1953, 1186.
[103] G. O. Aspinall, E. L. Hirst, E. G. V. Percival, and I. R. Williamson, *J.*, 1953, 3184.

structural investigations that the polysaccharide associated with yeast invertase [104] is identical with the mannan, yeast gum, obtained by the autolysis of yeast.[105] Iles mannan,[19] the polysaccharide extracted from the tubers of some *Amorphophallus* species, is a mixture of two linear polysaccharides, an α-1 : 4-linked polyglucosan resembling amylose and a β-1 : 4-linked glucomannan containing two mannose to every glucose residue. The galactomannan from Kentucky coffee bean [106] has the same general structure as guar and carob-bean galactomannans, containing a backbone of 1 : 4-linked D-mannopyranose residues with every fourth residue carrying at C_6 a D-galactopyranose residue as side-chain.

Glucosans.—Barley gum, the mixture of water-soluble polysaccharides isolated from barley grain, has been fractionated by precipitation with ammonium sulphate to give a lævorotatory glucosan free from pentosan.[6] Methylation has shown that this polysaccharide contains unbranched chains of β-D-glucopyranose residues with approximately equal proportions of 1 : 3- and 1 : 4-linkages; [107] the polysaccharide appears to be structurally related to lichenin. An evidently similar polysaccharide, the so-called or "lichenin" has been shown by periodate oxidation and partial acetolysis to possess 1 : 3- and 1 : 4-linked D-glucose units in the approximate ratio of 1 : 2.[108] The general character of pustulan, the polysaccharide obtained from the lichen *Umbilicaria pustulata*, has been indicated by the isolation from partial acid hydrolysis of a series of β-1 : 6-linked oligosaccharides; [109] the absence of oligosaccharides containing other linkages suggests that the polysaccharide is linear.

Fructosans.—Chemical evidence now indicates that many fructosans of both the inulin and the levan type contain terminal glucose residues linked as in sucrose, thus supporting the view that these polysaccharides are built up in the plant from sucrose by transfructosylation.[110, 111] The hydrolysis of methylated leafy cocksfoot levan [112] yields 1 : 3 : 4 : 6-tetra-*O*-methyl-D-fructose (4%), 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose (1·8%), and 1 : 3 : 4-tri-*O*-methyl-D-fructose (93·3%), showing the fructosan to be of levan type. D-glucose residues occur only as non-reducing end-groups, and it is probable that the majority of fructosan chains are terminated by sucrose-type linkages. The isolation of sucrose [113] from the partial hydrolysis of perennial rye-grass levan [114] provides definite proof of the existence of this terminal group in the polysaccharide. Perennial rye-grass also contains short-chain fructosans of chain length 5—10 having the same general structure.[115] Other workers,[116] however, find no glucose residues in perennial rye-grass levan

[104] J. A. Cifonelli and F. Smith, *J. Amer. Chem. Soc.*, 1955, **77**, 5682.
[105] W. N. Haworth, E. L. Hirst, and F. A. Isherwood, *J.*, 1937, 784; W. N. Haworth, R. L. Heath, and S. Peat, *J.*, 1941, 833.
[106] E. B. Larson and F. Smith, *J. Amer. Chem. Soc.*, 1955, **77**, 429.
[107] G. O. Aspinall and R. G. J. Telfer, *J.*, 1954, 3519.
[108] L. Acker, W. Diemair, and E. Samhammer, *Z. Lebensm.-Untersuch.*, 1955, **101**, 180; **102**, 225.
[109] B. Lindberg and J. McPherson, *Acta Chem. Scand.*, 1954, **6**, 985.
[110] S. A. Barker and E. J. Bourne, *Quart. Rev.*, 1953, **7**, 56.
[111] J. S. D. Bacon, *Ann. Reports*, 1953, **50**, 281.
[112] G. O. Aspinall, E. L. Hirst, E. G. V. Percival, and R. G. J. Telfer, *J.*, 1953, 3[illegible]
[113] G. O. Aspinall and R. G. J. Telfer, *J.*, 1955, 1106.
[114] R. A. Laidlaw and S. G. Reid, *J.*, 1951, 1830.
[115] V. D. Harwood, R. A. Laidlaw, and R. G. J. Telfer, *J.*, 1954, 2364.
[116] H. H. Schlubach and K. Holzer, *Annalen*, 1953, **578**, 207.

and postulate a difructose anhydride type of termination.[117] Studies of the degradation of fructosans in hot aqueous solution [113] emphasise the need for the utmost caution in the isolation of these extremely labile polysaccharides lest scission of the fructosan chain results in loss of the glucose-containing moiety. Methylation studies have shown that the fructosans from Kentucky blue,[118] red fescue,[119] and common fox-tail [120] grasses are of the levan type. Studies on the fructosans from the stems and ripening ears of the common cereals have been reviewed.[121]

Starch and Glycogen.—The fractionation of starch under conditions which minimise the possibility of degradation, particularly of the amylose, continues to attract much attention. The linear component appears to be especially susceptible to degradation, which may occur in at least two ways. It has been shown viscometrically that potato amylose is degraded in aqueous solution in the presence of oxygen; [122] this oxidative degradation, which is appreciable in neutral solution, occurs much more rapidly in alkali. Amylose is degraded by alkali in the absence of oxygen with the formation of a mixture of D-gluco*iso*saccharinic acids.[123] The fall in viscosity would be much less obvious if degradation proceeds solely by the "peeling" reaction [124] from the reducing end of the chain, than if random oxidation results in the formation of alkali-sensitive bonds in the middle of the chain. A method for the anærobic fractionation of starch, which depends on the preferential solubility of amylose in water, has been reported.[125] As a result of other investigations, it has been suggested that amylose and amylopectin occur naturally in chemical combination,[126] possibly through a phosphatide cross-link, and that preferential precipitation of the amylose with a complexing agent can only occur when the acid-labile linkage has been severed. The claim [127] that amylopectin can be purified by selective precipitation of the amylose with stearic acid has been refuted.[128]

The oxidation of starches by potassium metaperiodate has been studied in detail, and an accuracy of $\pm 0{\cdot}5$ glucose residue is claimed for unit-chain length determinations.[129] The repeating units of many starches have been determined by this method, and from parallel determinations of their amylose contents,[130] the average unit-chain lengths of the amylopectin components calculated. A valve microvoltmeter, which increases the accuracy of differential potentiometric titrations,[131] has been described and its use in studying the interaction of starches and other branched α-1 : 4-glucosans with iodine reported.[130] This sensitive method not only enables the amylose

[117] H. H. Schlubach and K. Hølzer, *Annalen*, 1953, **578**, 213.
[118] H. H. Schlubach and L. Gassmann, *ibid.*, 1953, **583**, 81.
[119] H. H. Schlubach and K. Holzer, *ibid.*, p. 88.
[120] H. H. Schlubach, K. Holzer, and L. Gassmann, *ibid.*, 1954, **587**, 107.
[121] H. H. Schlubach, *Experientia*, 1953, **9**, 230.
[122] R. T. Bottle, G. A. Gilbert, C. T. Greenwood, and K. N. Saad, *Chem. and Ind.*, 1953, 541.
[123] J. Kenner and G. N. Richards, *ibid.*, 1954, 1483.
[124] W. M. Corbett and J. Kenner, *J.*, 1955, 1431.
[125] H. A. Baum and G. A. Gilbert, *Chem. and Ind.*, 1954, 490.
[126] A. W. Bauer and E. Pacsu, *Textile Res. J.*, 1953, **23**, 853, 860, 864, 870.
[127] K. H. Meyer and G. C. Gibbons, *Helv. Chim. Acta*, 1950, **33**, 210.
[128] G. A. Gilbert, C. T. Greenwood, and F. J. Hybart, *J.*, 1954, 4454.
[129] D. M. W. Anderson, C. T. Greenwood, and E. L. Hirst, *J.*, 1955, 225.
[130] D. M. W. Anderson and C. T. Greenwood, *J.*, 1955, 3016.
[131] G. A. Gilbert and J. V. R. Marriott, *Trans. Faraday Soc.*, 1948, **44**, 84.

contents of starches to be accurately determined but also detects significant differences in iodine binding power between amylopectins and glycogens.

In a detailed chemical and physical study,[132] the starch from rubber seeds was found to contain 20% of amylose and an amylopectin of average unit-chain length 23 ± 1 glucose units, and in which the majority of branch points were through $C_{(6)}$. Starches of abnormally high amylose content and with amylopectins of increased unit-chain length have been isolated from wrinkled-seeded peas [133] and from a variety of maize.[134] Whilst the starch from smooth-seeded peas contained 35% of amylose (*i.e.*, more than normal) and an amylopectin of normal unit-chain length (25 units), that from wrinkled-seeded peas contained 66% of amylose and an amylopectin of chain length 36.[133] The abnormal maize starch contained *ca.* 50% of amylose and an amylopectin with a repeating unit of 36 glucose residues; the β-amylolysis limit of 58% for this amylopectin indicated an inner chain of 13 units (cf. 5—8 units for an average amylopectin),[135] and showed that both inner and outer chains were longer than usual. Structural studies of the starch from malted barley have indicated that malting results in the partial degradation of the outer chains of the amylopectin (26 ⟶ 18 units) with relatively little degradation of the amylose.[136]

Evidence that the inter-chain linkages in glycogens are only of the 1 : 6-type has been provided by the virtual absence of glucose in the hydrolysates of several periodate-oxidised glycogens,[137] and in the case of baker's yeast glycogen by " linkage analysis " in which the oligosaccharides formed on partial acid hydrolysis were shown to contain only α-1 : 4- and α-1 : 6-linkages. Both chemical and enzymic methods have been used in the investigation of the structure of baker's [138, 139] and brewer's [140] yeast glycogens. Similar methods have been used in the examination of an abnormal glycogen, from a case of von Geirke's disease, which had a unit-chain length of only 6 units.[141]

Algal Polysaccharides.—Recent work on the fine structure of laminarin has necessitated a modification of the view that this polysaccharide is composed solely of β-1 : 3-linked D-glucopyranose residues. In addition to the major product, laminaribiose, gentiobiose, 1-*O*-β-D-glucosyl-D-mannitol,[142] and 1-*O*-laminaribiosyl-D-mannitol [143] have been isolated on partial acid hydrolysis of the polysaccharide. It is evident, however, that not all laminarin molecules are terminated by mannitol residues as some of the polysaccharide is degraded on treatment with lime water; [124] it is probable that two closely-related molecular species are present. The mercaptolysis of algal polysaccharides has given evidence of 3 : 6-anhydrogalactose residues

[132] C. T. Greenwood and J. S. M. Robertson, *J.*, 1954, 3769.
[133] A. L. Potter, V. Silveira, R. M. McCready, and H. S. Owens, *J. Amer. Chem. Soc.*, 1953, **75**, 1335.
[134] I. A. Wolff, B. T. Hofreiter, P. R. Watson, W. L. Deatherage, and M. M. Masters, *ibid.*, 1955, **77**, 1654.
[135] D. J. Manners, *Quart. Rev.*, 1955, **9**, 73.
[136] G. O. Aspinall, E. L. Hirst, and W. McArthur, *J.*, 1955, 3075.
[137] D. J. Bell and D. J. Manners, *J.*, 1954, 1891.
[138] S. Peat, W. J. Whelan, and T. E. Edwards, *J.*, 1955, 355.
[139] D. H. Northcote, *Biochem. J.*, 1953, **53**, 348.
[140] D. J. Manners and Khin Maung, *J.*, 1955, 867.
[141] D. J. Manners, *J.*, 1954, 3527.
[142] S. Peat, W. J. Whelan, and H. G. Lawley, *Chem. and Ind.*, 1955, 35.
[143] S. Peat, W. J. Whelan, H. G. Lawley, and J. M. Evans, *Biochem. J.*, 1955, 61.

The mercaptolysis of agar thus yields the diethylmercaptals of D-galactose, DL-galactose, and 3 : 6-anhydro-L-galactose,[144] and of 3 : 6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactose (agarobiose).[145] The isolation from agar in 69·5% yield of agarobiose dimethylacetal and its methanolysis products [146] indicates that the agarobiose residue is the dominant repeating unit of this polysaccharide. On the other hand, the mercaptolysis of the polysaccharide from *Chondrus crispus* yields the diethylmercaptal of 3 : 6-anhydro-D-galactose.[147] The heterogeneous character of this polysaccharide has been indicated by a fractionation which gave κ-carrageenin, precipitated by potassium chloride, and λ-carrageenin.[148] κ-Carrageenin contains D-galactose, 3 : 6-anhydro-D-galactose, and sulphate groups in approximately equimolecular proportions, and the low consumption of periodate by the polysaccharide suggests that the D-galactose 4-sulphate residues are linked through positions 1 and 3.[149] λ-Carrageenin is composed mainly of D-galactose sulphate residues, only small quantities of the anhydro-sugar being present.[149] A re-investigation [150] of Floridean starch has failed to produce evidence for the presence of 1 : 3-linkages; the periodate oxidation of the polysaccharide and an examination of the thiosemicarbazide and *iso*nicotinhydrazide derivatives of the oxidised polysaccharide indicate that all the glucose residues are attacked by periodate.[63] The conversion of the main product of partial acetolysis of fucoidin into 2-*O*-L-fucopyranosyl-L-fucitol confirms the presence of 1 : 2-linked L-fucose residues in this polysaccharide.[151] A sulphated polysaccharide from *Ulva lactuca* [152] contains D-glucose, D-xylose, L-rhamnose, and D-glucuronic acid residues, and preliminary evidence as to its molecular structure has been obtained from methylation and periodate oxidation.

A complex acidic polysaccharide, containing D-glucose, D-xylose, D-galactose, L-rhamnose, L-arabinose, and glucuronic acid units, has been extracted from the fresh-water alga, *Anabena cylindrica*.[153] The cellulose from the alga *Chara* [154] has been examined; extraction of the alga with alkali yields a starch-like polysaccharide [155] contaminated by small amounts of a xylose-containing polysaccharide.

Plant Gums and Mucilages.—The occurrence of L-arabopyranose in addition to L-arabofuranose residues in plant gums has been demonstrated in the cases of cherry,[156] peach,[156] golden apple,[157] lemon,[158] and *Acacia karroo* [159] gums. In each case 3-*O*-β-L-arabopyranosyl-L-arabinose, previously isolated from larch ε-galactan,[96] was present amongst the products

[144] C. Araki and S. Hirase, *Bull. Chem. Soc. Japan*, 1953, **26**, 463.
[145] S. Hirase and C. Araki, *ibid.*, 1954, **27**, 105.
[146] C. Araki and S. Hirase, *ibid.*, p. 109.
[147] E. Percival, *Chem. and Ind.*, 1954, 1487; A. N. O'Neill, *J. Amer. Chem. Soc.*, 1955, **77**, 2837.
[148] D. A. I. Goring and E. G. Young, *Canad. J. Chem.*, 1955, **33**, 480.
[149] D. B. Smith, A. N. O'Neill, and A. S. Perlin, *ibid.*, p. 1352.
[150] P. O'Colla, *Proc. Roy. Irish Acad.*, 1953, **55**, *B*, 321.
[151] A. N. O'Neill, *J. Amer. Chem. Soc.*, 1954, **76**, 5074.
[152] J. W. E. Brading, M. M. T. Georg-Plant, and D. M. Hardy, *J.*, 1954, 319.
[153] C. T. Bishop, G. A. Adams, and E. O. Hughes, *Canad. J. Chem.*, 1954, **32**, 999.
[154] El S. Amin, *J.*, 1955, 281.
[155] *Idem, ibid.*, p. 282.
[156] P. Andrews, D. H. Ball, and J. K. N. Jones, *J.*, 1953, 4090.
[157] P. Andrews and J. K. N. Jones, *J.*, 1954, 4134.
[158] *Idem, J.*, 1955, 583.
[159] A. J. Charlson, J. R. Nunn, and A. M. Stephen, *J.*, 1955, 1428.

of partial acid hydrolysis; although this disaccharide can be formed as an acid reversion product from L-arabinose,[160] it is probable that in these cases its isolation has structural significance. Under similar conditions, 5-*O*-β-D-xylopyranosyl-L-arabinose has been isolated from peach [156] and cholla [156] gums, and 3-*O*-α-D-xylopyranosyl-L-arabinose from golden-apple gum.[157] Lemon gum also yields 4-*O*-(4-*O*-methyl-D-glucuronosyl)-L-arabinose on graded hydrolysis.[161]

Successive applications of Barry's degradation [162] have shown that gum arabic contains a central core of 1 : 3-linked D-galactopyranose residues; a similar conclusion has been reached from a study of the fragment of the degraded gum remaining after periodate oxidation and controlled hydrolysis to remove the cleaved aldobiouronic acid side-chains.[163] Several gums of the *Acacia* genus, namely *A. senegalensis* (gum arabic),[164] *A. mollissima*,[165] *A. pycnantha*,[166] *A. cyanophylla*,[167] and *A. karroo*,[159] contain the same constituent sugars, although in different proportions; all yield the same aldobiouronic acid, 6-*O*-β-D-glucuronosyl-D-galactose, on partial hydrolysis. In the case of *A. karroo* gum, a second aldobiouronic acid, 4-*O*-α-D-glucuronosyl-D-galactose, was isolated.[159] The similarity of *A. pycnantha* [166] and *A. cyanophylla* [167] gums to gum arabic is emphasised by the isolation of 3-*O*-D-galactopyranosyl-D-galactose [168] and 3-*O*-α-D-galactopyranosyl-L-arabinose,[169] respectively, from the products of partial hydrolysis. Gum ghatti [170] resembles damson [171] and cherry [172] gums in respect of its constituent sugars, L-arabinose, D-galactose, D-mannose, xylose and D-glucuronic acid, and in yielding the same aldobiouronic acid, 2-*O*-β-D-glucuronosyl-D-mannose on partial hydrolysis, but the isolation also of 6-*O*-β-D-glucuronosyl-D-galactose suggests some similarity with the gums of the *Acacia* group. 4-*O*-(4-*O*-Methyl-α-D-glucuronosyl)- and 6-*O*-(4-*O*-methyl-β-D-glucuronosyl)-D-galactose have been isolated from gum myrrh,[173] and 4-*O*-D-glucuronosyl-D-galactose and 3-*O*-D-glucuronosyl-D-galactose from Neem gum [174] and Ketha gum,[175] respectively. The partial methanolysis of methylated sapote gum yields the methyl glycosides of 3-*O*-methyl-D-xylose, 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3 : 4-tri-*O*-methyl-L-arabinose, and 3 : 4-di-*O*-methyl-D-glucuronic acid, together with the methyl glycosides of the partially methylated aldobiouronic acids, 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose and 2-*O*-(3 : 4-di-*O*-methyl-D-glucuronosyl)-

[160] D. H. Ball, J. K. N. Jones, and W. H. Nicholson, Amer. Chem. Soc. Meeting, Minneapolis, Sept., 1955, Abs. Papers, 7D.
[161] P. Andrews and J. K. N. Jones, *J.*, 1954, 1724.
[162] T. Dillon, D. F. O'Ceallachain, and P. O'Colla, *Proc. Roy. Irish Acad.*, 1953, **55**, *B*, 331; 1954, **57**, *B*, 31.
[163] F. Smith and D. Spriestersbach, Amer. Chem. Soc. Meeting, Minneapolis, Sept., 1955, Abs. Papers, 15D.
[164] S. W. Challinor, W. N. Haworth, and E. L. Hirst, *J.*, 1931, 258.
[165] A. M. Stephen, *J.*, 1951, 646.
[166] E. L. Hirst and A. S. Perlin, *J.*, 1954, 2622.
[167] A. J. Charlson, J. R. Nunn, and A. M. Stephen, *J.*, 1955, 269.
[168] J. Jackson and F. Smith, *J.*, 1940, 79.
[169] F. Smith, *J.*, 1939, 744.
[170] G. O. Aspinall, E. L. Hirst, and A. Wickstrom, *J.*, 1955, 1160.
[171] E. L. Hirst and J. K. N. Jones, *J.*, 1938, 1174.
[172] J. K. N. Jones, *J.*, 1939, 558.
[173] J. K. N. Jones and J. R. Nunn, *J.*, 1955, 3001.
[174] S. Mukherjee and H. C. Srivasta, *J. Amer. Chem. Soc.*, 1955, **77**, 422.
[175] G. P. Mathur and S. Mukherjee, *J. Sci. Ind. Res.* (*India*), 1954, **13**, *B*, 452.

3-*O*-methyl-D-xylose.[176] In contrast to these glucuronic acid-containing gums, *Cochlospermum gossypium* gum [177] contains residues of D-galacturonic acid, in addition to L-rhamnose and D-galactose. The hydrolysis products from the methylated gum, together with the mixture of aldobiouronic acids, 2-*O*-D-galacturonosyl-L-rhamnose and 4-*O*-D-galacturonosyl-D-galactose, obtained on partial hydrolysis, indicate a highly branched structure.

A re-investigation of the mucilage from *Plantago arenaria* seeds [178] still leaves doubt concerning the homogeneity of the polysaccharide. Although 2-*O*-α-D-galacturonosyl-L-rhamnose was isolated from the partial hydrolysis of the mucilage, the hydrolysis of the methylated polysaccharide gave a complex mixture of methyl ethers of D-xylose, L-arabinose, and D-galactose, but no methylated derivatives of D-galacturonic acid or L-rhamnose could be isolated. Partial hydrolysis of okra mucilage [179] yielded 4-*O*-α-D-galactopyranosyl-D-galactose and 2-*O*-α-D-galacturonosyl-L-rhamnose. An acidic polysaccharide isolated from the juice of ripe grapes consisted of residues of D-galactose, D-mannose, L-arabinose, L-rhamnose, and D-galacturonic acid, and yielded 2-*O*-α-D-galacturonosyl-L-rhamnose on partial hydrolysis.[180]

Polysaccharides synthesised by Micro-organisms.—Further structural examinations of dextrans have shown that these polysaccharides have backbones of α-1 : 6-linked D-glucopyranose residues, but that they differ considerably in their degrees of branching and also in the nature of the branch points. Thus, *Betacoccus arabinosaceous* normally synthesises a branched dextran with a repeating unit of 6—7 glucose residues and with branching through $C_{(3)}$.[181] The same organism, when grown in a magnesium-deficient medium, elaborates a much less highly branched polysaccharide of the same general type with a unit-chain length of 40—50.[182] A large number of dextrans, synthesised by different strains of *Leuconostoc mesenteroides*, have been examined by periodate oxidation,[183, 184] and the proportions of 1 : 6-, 1 : 4-, and 1 : 3-linkages determined. The results obtained from the quantitative analysis of the products (glucose, glycerol, and erythritol) of hydrolysis of the polyol, isolated from the catalytic reduction of the periodate-oxidised polysaccharides,[1] were in reasonable agreement with those calculated from the titrimetric determinations of periodate consumed and formic acid released during the oxidation.[183] Methylation has shown that the levans formed by *Pseudomonas prunicola*, Wormald and *Bacillus subtilis* BG2 Fl are branched molecules, with repeating units of 9—10 2 : 6-linked β-D-fructofuranose residues and with 2 : 1-linkages at the branch points.[185] A similar levan, but of shorter average chain length (8—9 units), has been isolated from *B. polymyxa*.[186]

[176] E. V. White, *J. Amer. Chem. Soc.*, 1953, **75**, 257, 4692; 1954, **76**, 4906.
[177] E. L. Hirst and S. Dunstan, *J.*, 1953, 2332.
[178] E. L. Hirst, E. G. V. Percival, and C. B. Wylam, *J.*, 1954, 189.
[179] R. L. Whistler and H. E. Conrad, *J. Amer. Chem. Soc.*, 1954, **76**, 1673, 3544.
[180] W. Büchi and H. Deuel, *Helv. Chim. Acta*, 1954, **37**, 1392.
[181] S. A. Barker, E. J. Bourne, G. T. Bruce, W. B. Neely, and M. Stacey, *J.*, 1954, 2395.
[182] S. A. Barker, E. J. Bourne, A. E. James, W. B. Neely, and M. Stacey, *J.*, 1955, 2096.
[183] J. W. Sloan, B. H. Alexander, R. L. Lohmar, I. A. Wolff, and C. E. Rist, *J. Amer. Chem. Soc.*, 1954, **76**, 4429.
[184] J. C. Rankin and A. Jeanes, *ibid.*, p. 4435.
[185] D. J. Bell and R. Dedonder, *J.*, 1954, 2866.
[186] D. Murphy, *Canad. J. Chem.*, 1952, **30**, 872.

Several polysaccharides of the amylopectin–glycogen type have been isolated from micro-organisms. The polysaccharides synthesised by the holotrichously ciliated protozoa present in the sheep's rumen [187] and by *Cyclopostium* found in the colon and cæcum of a horse [188] contain unit chains of 22—23 α-1 : 4-linked D-glucose residues joined by 1 : 6-linkages. The polysaccharides from the protozoa *Trichomonas fœtus* and *T. gallinea*,[189] and from *Bacillus megatherium* [190] contain shorter unit chains of 15, 9, and 10—11 residues, respectively.

Serological cross reactions have been used in the comparison of the acidic capsular polysaccharide from *Azotobacter chroococcum* [191] and the Type II *Pneumococcus* specific polysaccharide [192] with polysaccharides whose main structural features are known. Methylation has shown that the *A. chroococcum* polysaccharide contains D-glucose and D-galactose residues in the ratio of 3 : 1, together with a small proportion of D-glucuronic acid residues. From similar experiments, it is clear that the Type II *Pneumococcus* polysaccharide is highly branched and contains L-rhamnose, D-glucose, and D-glucuronic acid residues in the approximate ratio of 7 : 1 : 3; whilst the detailed structure is not yet known, the molecule must contain chains of 1 : 3-linked L-rhamnopyranose residues. Two serologically active polysaccharides have been isolated from *Bacillus anthracis*; [193] one of the polysaccharides is a mannan in which the residues are 1 : 4-linked, whilst the other contains D-galactose and *N*-acetyl-D-glucosamine units in the ratio of 2 : 1.

L-Fucose is a constituent sugar of three widely different polysaccharides. The capsular polysaccharide from *Pseudomonas fluorescens*, strain II, yields on hydrolysis D-glucose, D-glucosamine, L-fucose, a crystalline disaccharide composed of glucose and fucose, and a crystalline tetrasaccharide composed of a glucose, a glucosamine, and two fucose residues.[194] The extracellular polysaccharide from *Mucor racemosus* [195] is composed of residues of D-galactose, D-mannose, L-fucose, and D-glucuronic acid; it is probable that all the fucose residues occur in the furanose form in terminal positions as they are all attacked by periodate and are all released on autohydrolysis of the polysaccharide. An extracellular polysaccharide from *Aerobacter aerogenes* is composed of D-glucose (50%), L-fucose (10%), and unidentified uronic acid residues (29%).[196]

Mucopolysaccharides.—Although much work has been carried out during recent years on this group of polysaccharides, knowledge of their detailed chemical structure is still limited. Some aspects of the chemistry of these substances, including their relation to proteins in protein-carbohydrate complexes, formed a part of the Faraday Society Discussion on "The

[187] G. Forsyth and E. L. Hirst, *J.*, 1953, 2132.
[188] G. Forsyth, E. L. Hirst, and A. E. Oxford, *J.*, 1953, 2030.
[189] D. J. Manners and J. F. Ryley, *Biochem. J.*, 1955, **59**, 369.
[190] C. Barry, R. Cavard, G. Milhaud, and J. P. Aubert, *Ann. Inst. Pasteur*, 1953, 84, 605.
[191] G. J. Lawson and M. Stacey, *J.*, 1954, 1925.
[192] K. Butler and M. Stacey, *J.*, 1955, 1537.
[193] J. E. Cave-Brown-Cave, E. J. S. Fry, H. S. El Khadem, and H. N. Rydon, *J.*, 1954, 3866.
[194] R. G. Eadon and R. Dedonder, *Compt. rend.*, 1955, **241**, 579.
[195] L. Hough and M. B. Perry, *Biochem. J.*, 1955, **61**, viii.
[196] J. F. Wilkinson, W. F. Dudman, and G. O. Aspinall, *Biochem. J.*, 1955, **59**, 44

Physical Chemistry of Proteins."[197] The structure of chondrosin, the disaccharide obtained from cartilage chondroitinsulphuric acid,[198] has been established as 2-amino-2-deoxy-3-*O*-(β-D-glucopyranuronosyl)-D-galactose by degradation and reduction to 2-*O*-(β-D-glucopyranosyl)-D-lyxitol, whose structure was proved by periodate oxidation.[199] A similar conclusion was reached from a study of the periodate oxidation of *N*-acetylchondrosin ethyl ester.[200] Chondroitin,[201] a mucopolysaccharide isolated from bovine cornea, has a very low sulphate content and also yields chondrosin on hydrolysis. The disaccharide isolated from the partial acid hydrolysis of hog gastric mucin with blood group A activity has been identified as 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose by periodate oxidation,[202, 203] and by oxidative degradation (with ninhydrin) to,[203] and synthesis from,[204] 3-*O*-β-D-galactopyranosyl-D-arabinose, and also by comparison with the synthetic disaccharide.[205] Hydrolysis of the periodate-oxidised hog-mucin polysaccharide after bromine oxidation yielded tartronic acid, D-glyceric acid, and D-glucosamine hydrochloride, and so showed that the galactose residues are linked through $C_{(2)}$.[206] Periodate oxidations of the polysaccharide and its degradation products suggest that the small proportion of L-fucose residues (<10%) present in the molecule replace some of the D-galactose residues in the basic disaccharide repeating unit, probably near the ends of the chains.[207]

G. O. A.
J. C. P. S.

11. AMINO-ACIDS, PEPTIDES, AND PROTEINS.

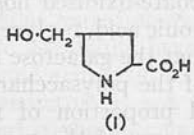
Natural Amino-acids.—A new attempt has been made to discover the ultimate origin of natural amino-acids. In a remarkable paper [1] evidence has been presented that a wide variety of amino-acids is formed when a mixture of methane, ammonia, hydrogen, and water are subjected to the combined action of a silent electrical discharge and a high-frequency spark. In contrast with the results of earlier attempts to reproduce the synthesis of the raw materials of life *ab initio*, the products in this case were appreciable in quantity and identified with certainty, while proof that microbiological processes were not involved was adequate. Glycine, α- and β-alanine, sarcosine, and α-aminobutyric acid were among the thirty or more ninhydrin-active compounds separated.

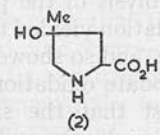
A considerable number of new natural amino-acids has been reported, many of which occur in the free state. Recent surveys have been made of

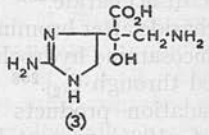
[197] *Discuss. Faraday Soc.*, 1953, **13**, pp. 245—287.
[198] E. A. Davidson and K. Meyer, *J. Amer. Chem. Soc.*, 1954, **76**, 5686.
[199] *Idem, ibid.*, 1955, **77**, 4796.
[200] H. Masamune, Z. Yoshizawa, and M. Maki, *Tohuku J. Exp. Med.*, 1951, **55**, 47.
[201] E. A. Davidson and K. Meyer, *J. Biol. Chem.*, 1954, **211**, 605.
[202] Z. Yoshizawa, *Tohuku J. Exp. Med.*, 1950, **52**, 111.
[203] F. Zilliken, P. N. Smith, R. M. Tomarelli, and P. Gyorgy, *Arch. Biochem. Biophys.*, 1955, **54**, 398.
[204] Z. Yoshizawa, *Tohuku J. Exp. Med.*, 1950, **52**, 145.
[205] R. Kuhn and W. Kirschenlohr, *Chem. Ber.*, 1954, **87**, 1547.
[206] Z. Yoshizawa, *Tohuku J. Exp. Med.*, 1951, **54**, 129.
[207] H. Masamune and Z. Yoshizawa, *ibid.*, 1954, **60**, 135.
[1] S. L. Miller, *J. Amer. Chem. Soc.*, 1955, **77**, 2351.

free amino-acids both in plant and animal tissues.[2] The biochemical significance of much recent work is outside the scope of this Report which will be concerned with only a selection of papers published in 1954 and 1955.

The methylsulphonium analogue of methionine has been obtained in two laboratories [3] and appears to be of wide occurrence in green plants. It is more active than methionine as an antagonist of the toxicity of sulphanilamide for certain micro-organisms. A substantial proportion of the organic sulphur of cabbage is believed to be present as one of the diastereoisomeric forms of L-*S*-methylcysteine sulphoxide (β-methylsulphinylalanine).[4] Besides the occurrence of γ-methylproline in young apple fruit, a further new proline derivative has been observed in both the fruit and the twigs of apple trees.[5] It is believed [6] to be a hydroxymethylproline, but it is not as yet possible to distinguish between the two possibilities (1) and (2).


(1)


(2)


(3)

Roseothricin, besides containing 3 : 6-diaminohexanoic acid,[7] yields an amino-acid "roseonine" to which the structure (3) has been assigned. Permanganate oxidation of the material gave guanidine and a small quantity of glycine; one mol. of periodate was consumed quickly, producing formaldehyde and ammonia, and, after twenty hours, a further mol. of oxidant was used. This behaviour is similar to that of serine and was regarded as support for structure (3). It seems to the Reporter, however, that the structure needs further consideration in view of the fact that the pK_a' value is lower than that of *iso*serine.

A previously unidentified peak in ion-exchange chromatograms of human urine has been shown to be due to L-3-methylhistidine,[9] which has been obtained also, together with other methylhistidines, by methylation of histidine and is distinguished from 1-methylhistidine by chromatography on Dowex-50 resin and by its infrared spectrum.

The presence of α-aminopimelic acid [10] and of α-amino-γ-hydroxypimelic acid [11] in hydrolysates of nerve proteins has been claimed, but α-aminobutyric acid, previously obtained therefrom,[12] has now been shown to arise by decomposition of threonine hydrochloride and serine hydr

[2] N. Grobbelaar, J. K. Pollard, and F. C. Steward, *Nature*, 1955, **175**, 703; A. Virtanen, *Angew. Chem.*, 1955, **67**, 381; W. H. Stein and S. Moore, *J. Biol. Chem.*, 1954, **211**, 915; H. H. Tallan and S. Moore, *ibid.*, p. 927.

[3] R. A. McRorie, G. L. Sutherland, M. S. Lewis, A. D. Barton, M. R. Glazener, and W. Shine, *J. Amer. Chem. Soc.*, 1954, **76**, 115; F. Challenger and B. J. Haywood, *Biochem. J.*, 1954, **58**, iv.

[4] R. L. M. Synge and J. C. Wood, *ibid.*, 1955, **60**, xv.

[5] A. C. Hulme and W. Arthington, *Nature*, 1954, **173**, 588; A. C. Hulme, *ibid.*, 1954, **174**, 1055; G. Urbach, *ibid.*, 1955, **175**, 170.

[6] A. C. Hulme and F. G. Steward, *ibid.*, p. 171.

[7] K. Nakanishi, T. Ito, M. Ohashi, I. Morimoto, and Y. Hirata, *Bull. Chem. Soc. Japan*, 1954, **27**, 539.

[8] K. Nakanishi, T. Ito, and Y. Hirata, *J. Amer. Chem. Soc.*, 1954, **76**, 2845.

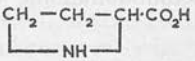
[9] H. H. Tallan, W. H. Stein, and S. Moore, *J. Biol. Chem.*, 1954, **206**, 825.

[10] A. I. Virtanen and A.-M. Berg, *Acta Chem. Scand.*, 1954, **8**, 1085.

[11] A. I. Virtanen, E. Uksila, and E. J. Matikkala, *ibid.*, p. 1091.

[12] K. Heyns and W. Walter, *Z. physiol. Chem.*, 1952, **289**, 85.

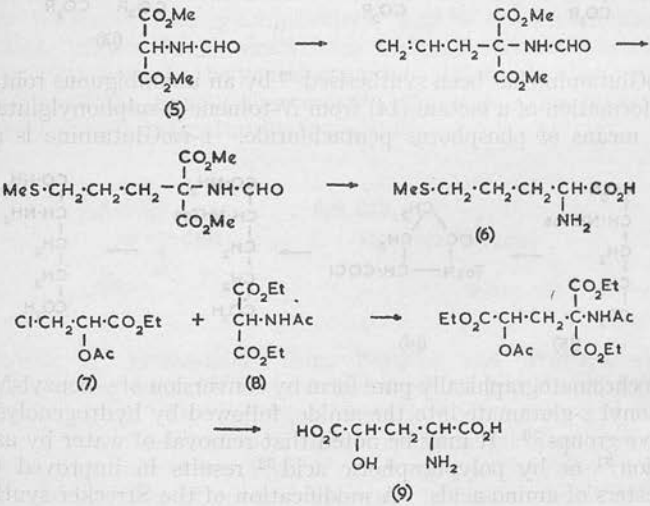
chloride.[13] An imino-acid from species of *Liliaceæ* has been identified[14] as an azetidine derivative (4). The tentative identification of γ-methylglutamic acid and γ-hydroxy-γ-methylglutamic acid in *Phyllitis scolopendrium*[15] and of γ-hydroxyglutamic acid in *Phlox decussata*[16] is to be



(4)

contrasted with the finding that Dakin's so-called β-hydroxyglutamic acid[17] consists mainly of a mixture of glutamic and aspartic acid.[18] It is also of interest that γ-methyleneglutamine, first reported as a constituent of the peanut plant,[19] and γ-methyleneglutamic acid have been recognised in the tulip bulb[20] and in hops.[21] The structures of the natural materials have been confirmed by synthesis.[22]

Synthetic Amino-acids.—Acylaminomalonic esters, for which an improved preparation is available,[23] have been used in a large number of the

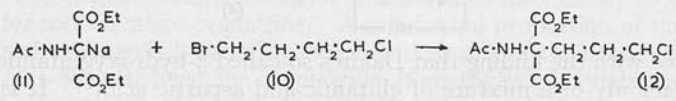


(5) (6) (7) (8) (9)

syntheses of amino-acids described in the period under review. DL-Homomethionine has been obtained[24] by the route (5) ⟶ (6) and DL-γ-hydroxyglutamic acid[25] by route (7) + (8) ⟶ (9). The latter product was also

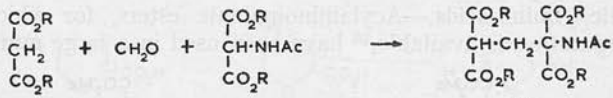
[13] K. Heyns and W. Walter, *Z. physiol. Chem.*, 1953, **294**, 111.
[14] A. I. Virtanen and P. Linko, *Acta Chem. Scand.*, 1955, **9**, 551; L. Fowden, *Nature*, 1955, **176**, 347; A. I. Virtanen, *ibid.*, p. 984; *Angew. Chem.*, 1955, **67**, 619.
[15] A. I. Virtanen and A.-M. Berg, *Acta Chem. Scand.*, 1955, **9**, 553.
[16] A. I. Virtanen and P. K. Hietala, *ibid.*, p. 175.
[17] H. D. Dakin, *Biochem. J.*, 1918, **12**, 290; 1919, **13**, 398.
[18] C. E. Dent and D. I. Fowler, *ibid.*, 1954, **56**, 54.
[19] J. Done and L. Fowden, *ibid.*, 1951, **49**, xx; 1951, **51**, 541.
[20] R. M. Zacharius, J. K. Pollard, and F. C. Steward, *J. Amer. Chem. Soc.*, 1954, **76**, 1961.
[21] G. Harris, *Chem. and Ind.*, 1954, 244.
[22] P. C. Wailes, M. C. Whiting, and L. Fowden, *Nature*, 1954, **174**, 130; P. C. Wailes and M. C. Whiting, *J.*, 1955, 3636.
[23] H. Hellmann and F. Lingens, *Z. physiol. Chem.*, 1954, **297**, 283.
[24] A. Kjaer and S. Wagner, *Acta Chem. Scand.*, 1955, **9**, 721.
[25] L. Benoiton and L. P. Bouthillier, *Canad. J. Chem.*, 1955, **33**, 1473.

obtained by bromination of α-phthalimidoglutaric anhydride, and its structure was confirmed by the fact that the material obtained by either route was unaffected by periodate. DL-Lysine has been synthesised by making use of the fact that 1-bromo-4-chlorobutane (10) reacts [26] with the sodio-derivative (11) to give the acetamidomalonic ester (12), and a further



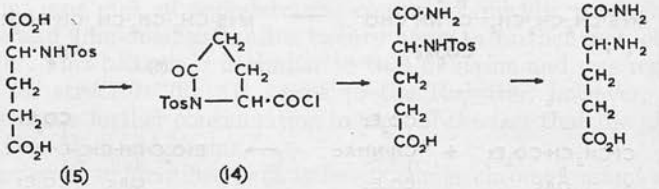
(11) (10) (12)

interesting adaptation [27] of this type of synthesis is the formation of glutamic acid through the intermediate (13). Glutamic acid has also been obtained from ethyl α-bromoglutarate.[28]



(13)

L-*iso*Glutamine has been synthesised [29] by an unambiguous route involving the formation of a lactam (14) from *N*-toluene-*p*-sulphonylglutamic acid (15) by means of phosphorus pentachloride. L-*iso*Glutamine is also pro-



(15) (14)

duced in chromatographically pure form by conversion of γ-benzyl-*N*-benzyloxycarbonyl L-glutamate into the amide, followed by hydrogenolysis of the protective groups.[30] It may be noted that removal of water by azeotropic distillation [31] or by polyphosphoric acid [32] results in improved yields of benzyl esters of amino-acids. A modification of the Strecker synthesis has been used for the preparation of DL-α-methylglutamic acid, which acts as an inhibitor of the synthesis and utilisation of glutamine.[33]

Other innovations include the synthesis of homologues of glutamic acid, methionine, and diaminopimelic acid *via* the hydantoins,[34] and the production of α-amino-acids by interaction of α-keto-aldehydes with ammonium salts in the presence of thiols.[35]

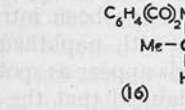
Stereochemical Relations.—An extensive study of the optical rotatio

[26] M. Servigne and E. Szarvasi, *Compt. rend.*, 1954, **238**, 1595.
[27] H. Hellmann and F. Lingens, *Angew. Chem.*, 1954, **66**, 201.
[28] G. Paris, R. Gaudry, and L. Berlinguet, *Canad. J. Chem.*, 1955, **33**, 1724.
[29] J. M. Swan and V. Du Vigneaud *J. Amer. Chem. Soc.*, 1954, **76**, 3110.
[30] M. Kraml and L. P. Bouthillier, *Canad. J. Chem.*, 1955, **33**, 1630.
[31] J. D. Cipera and R. V. V. Nicholls, *Chem. and Ind.*, 1955, 16.
[32] B. F. Erlanger and R. M. Hall, *J. Amer. Chem. Soc.*, 1954, **76**, 5781.
[33] A. E. Gal, S. Avakian, and G. J. Martin, *ibid.*, p. 4181.
[34] K. Pfister, W. J. Leanza, J. P. Conbere, H. J. Becker, A. R. Matzuk, and I Rogers, *ibid.*, 1955, **77**, 697.
[35] T. Wieland, J. Franz, and G. Pfleiderer, *Chem. Ber.*, 1955, **88**, 641.

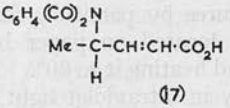
of amino-acids has been made and it is claimed that reliable conclusions can be drawn from optical data concerning the structures both of amino-acids containing one asymmetric centre, and of diastereoisomeric amino-acids.[36] Diastereoisomers of amino-acids such as hydroxylysine and *iso*leucine are now conveniently separated by ion-exchange chromatography.[37]

X-Ray studies have shown that (the usual convention being followed) the amino-group of D(−)-*iso*leucine is *cis* with respect to the methyl group.[38] It follows from this that, since L-*iso*leucine and D-*alloiso*leucine on treatment with ninhydrin give (+)-α-methylbutyraldehyde, and D-*iso*leucine and L-*alloiso*leucine give the lævorotatory aldehyde, the configurations of all four isomeric *iso*leucines are known.[39] It is interesting to note that *O*-methylthreonine acts as a competitive inhibitor for the incorporation of radioactive *iso*leucine into proteins, a fact which is in agreement with the existence of related configurations at both the asymmetric centres of the amino-acids.[40]

A method which may well be applicable to the determination of the configurations of diastereoisomeric amino-acids is exemplified by the conversion of L-alanine into γ-aminovaleric acid.[41] L-α-Phthalimidopropaldehyde (16), previously obtained from L-alanine, was converted by a Doebner condensation into the pent-2-enoic acid (17) which, after hydrogenation and hydrolysis, gave (+)-γ-aminovaleric acid. This aminovaleric acid is therefore related structurally to L-alanine.



(16)



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Preparation of Amino-acids from Peptides and Proteins.—Various modifications of the methods of acid hydrolysis of peptides and proteins have been introduced. Dissolution of tissue in 85% formic acid followed by addition of 2N-hydrochloric acid results in liberation of all amino-acids, except tryptophan, within two hours.[42] It is agreed that different peptide bonds are split at different rates,[43] but there appear to be differences of opinion as to the mechanism of the hydrolysis.[44] The preferential liberation of threonine and serine recorded by Elliott [45] has been observed also in the hydrolysis of wheat gluten under the conditions used by Elliott,[46] and also

[36] M. Winitz, S. M. Birnbaum, and J. P. Greenstein, *J. Amer. Chem. Soc.*, 1955, **77**, 716; M. C. Otey, J. P. Greenstein, M. Winitz, and S. M. Birnbaum, *ibid.*, p. 3112.
[37] K. A. Piez, *J. Biol. Chem.*, 1954, **207**, 77; P. B. Hamilton and R. A. Anderson, *ibid.*, 1955, **213**, 249.
[38] J. Trommel and J. M. Bijvoet, *Acta Cryst.*, 1954, **7**, 703.
[39] W. S. Fones, *J. Amer. Chem. Soc.*, 1954, **76**, 1377.
[40] M. Rabinovitz, M. E. Olsen, and D. M. Greenberg, *ibid.*, 1955, **77**, 3109.
[41] K. Balenović and D. Cerar, *J.*, 1955, 1631.
[42] S. U. Gurnani, U. S. Kumta, and M. B. Sahasrabudhe, *Biochim. Biophys. Acta*, 1955, **16**, 553.
[43] R. Hirohata, Y. Kanda, M. Nakamura, N. Izumiya, A. Nagamatsu, T. Ono, S. Fujii, and M. Kimitsuki, *Z. physiol. Chem.*, 1953, **295**, 368.
[44] R. J. L. Martin, *Nature*, 1955, **175**, 771.
[45] D. F. Elliott, *Biochem. J.*, 1952, **50**, 542.
[46] L. Wiseblatt, L. Wilson, and W. B. McConnell, *Canad. J. Chem.*, 1955, **33**, 1295.

during the hydrolysis of insulin[47] by 10·5N-hydrochloric acid at 0°. Hydrolysis of peptide bonds with acidic resins is being used increasingly. Dowex-50 is preferred,[48] but it is found that prolonged treatment with water causes appreciable breakdown of this resin, with liberation of sulphuric acid and some brown material, particularly with high degrees of cross-linking of the polymer.[49] Temperature is an important factor, and the method is unsuitable in certain cases such as the hydrolysis of insulin.[50]

During the alkaline fission of peptide bonds, a secondary reaction may occur whereby an *N*-terminal glycine residue reacts reversibly with an aldehyde to give a hydroxyamino-acid or, conversely, a terminal hydroxyamino-acid may be degraded to an aldehyde and glycine.[51] A new reaction has been reported which may prove useful in the degradation of peptides and proteins.[52]

Most developments in the isolation of amino-acids from protein hydrolysates concern partition, ion-exchange, or electrophoretic techniques. However, it is useful to note that methionine may be isolated from hydrolysates of casein and zein in 65% and 85% yield respectively by conversion into the methylsulphonium derivative which is precipitated as the phosphotungstate.[53] Sublimation is possible with a number of amino-acids and the technique should find considerable use in the purification of labelled materials.[54]

Partition Chromatography, Ion-exchange Chromatography, and Ionography of Amino-acids.—Various minor modifications in the analysis of amino-acid mixtures by partition chromatography have been introduced. Amino-acids are located on paper by spraying it with naphthaquinone sulphonic acid and heating it to 60°. The amino-acids appear as spots which fluoresce strongly in ultraviolet light,[55] and it is claimed that the method is more sensitive than the ninhydrin technique. Modifications in technique now largely eliminate errors in the quantitative determination of amino-acids on paper chromatograms by the ninhydrin method,[56] and improvements in the copper method have also been suggested.[57]

Ion-exchange chromatography is probably more widely applicable to the separation and isolation of amino-acids; and, of the many studies of the amino-acid composition of proteins which have come to the notice of the Reporter during the last two years, over half have employed ion-exchange chromatography. Zeokarb-225 can be used [58] instead of Dowex-50, as the original work of Moore and Stein.[59] It is recommended that "water regain" as defined by Pepper [60] be used instead of the degree of cross-

[47] G. L. Mills, *Biochem. J.*, 1954, **56**, 230.
[48] J. R. Whitaker and F. E. Deatherage, *J. Amer. Chem. Soc.*, 1955, **77**, 3360.
[49] A. S. Dixon, *Biochem. J.*, 1955, **59**, xii; **60**, 165.
[50] J. C. Paulson and F. E. Deatherage, *J. Amer. Chem. Soc.*, 1954, **76**, 6198.
[51] T. Wieland and K. Dose, *Angew. Chem.*, 1954, **66**, 781.
[52] K. Heyns and K. Stange, *Z. Naturforsch.*, 1955, **10**b, 129.
[53] N. F. Floyd and T. F. Lavine, *J. Biol. Chem.*, 1954, **207**, 119.
[54] D. Gross and G. Grodsky, *J. Amer. Chem. Soc.*, 1955, **77**, 1678.
[55] E. Kofranyi, *Z. physiol. Chem.*, 1955, **299**, 129.
[56] W. Gerok, *ibid.*, p. 112.
[57] H. Boser, *ibid.*, 1954, **296**, 10.
[58] P. N. Campbell, S. Jacobs, T. S. Work, and T. R. E. Kressman, *Chem. and* 1955, 117.
[59] S. Moore and W. H. Stein, *J. Biol. Chem.*, 1951, **192**, 663.
[60] K. W. Pepper, *J. Appl. Chem.*, 1951, **1**, 124.

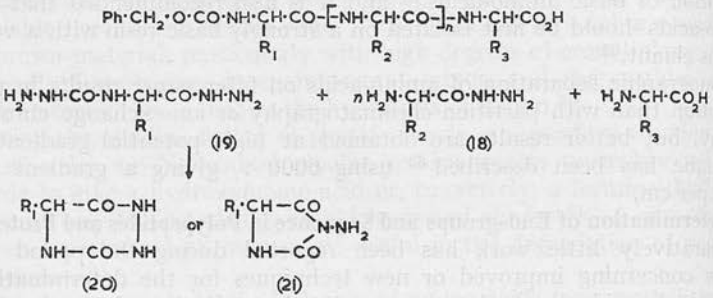
linking to designate a particular resin since this quantity can readily be measured. Minor modifications in technique are advisable for effective resolution of basic amino-acids [61] and it is also recommended that acidic amino-acids should be first isolated on a strongly basic resin with a volatile acid as eluant.[62]

Ionographic separation of amino-acids on filter paper results in poorer resolution than with partition chromatography or ion-exchange chromatography, but better results are obtained at high potential gradients. A technique has been described [63] using 6000 v, giving a gradient up to 130 v per cm.

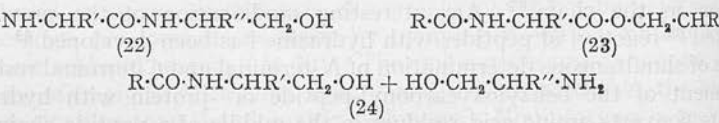
Determination of End-groups and Sequence in Polypeptides and Proteins.—Comparatively little work has been reported during the period under review concerning improved or new techniques for the determination of *N*-terminal residues. It has been pointed out [64] that determination of *N*-terminal residues by the fluorodinitrobenzene method and of *C*-terminal residues by using carboxypeptidase may lead to contradictory results. The large number of papers dealing with the determination of *C*-terminal residues is indicative of the fact that a wholly satisfactory method has yet to be found. The 2-thiohydantoin method still receives considerable attention,[65] but serine and proline are now added to the list of *C*-terminal groups which vitiate the method. Anodic oxidation of *C* terminal residues according to Boissonnas [66] is found to be unsatisfactory with peptides containing phenylalanine or tyrosine since these amino-acids are destroyed irrespective of their position in the chain.[67] An interesting modification of the previously reported [68] reaction of peptides with hydrazine has been developed.[69] This allows of simultaneous determination of *N*-terminal and *C*-terminal residues. Treatment of the benzyloxycarbonyl-peptide or -protein with hydrazine hydrate converts amino-acid residues in the middle of a peptide chain into amino-acid hydrazides (18), and the *N*-terminal residue is converted into the dihydrazide (19), whereas *C*-terminal amino-acid is liberated as such. The dihydrazides are converted into what may be either a triazine (20) or an aminohydantoin (21), and the products are separated chromatographically. The method is not satisfactory for the detection of glycine, serine, or cysteine as *N*-terminal residues, since compounds of type (20) or (21) are not formed; difficulties also arise with glutamyl-peptides. Insulin has been subjected to ammonolysis in liquid ammonia at 120° which converts all residues into amides except the *C*-terminal residue which is recovered as the free amino-acid.[70] *C*-Terminal groups have also been converted by acetic anhydride

[61] P. B. Hamilton and R. A. Anderson, *J. Biol. Chem.*, 1954, **211**, 95.
[62] C. H. W. Hirs, S. Moore, and W. H. Stein, *J. Amer. Chem. Soc.*, 1954, **76**, 6063.
[63] D. Gross, *Nature*, 1955, **176**, 72.
[64] M. Rovery and P. Desnuelle, *Bull. Soc. Chim. biol.*, 1954, **36**, 95.
[65] A. L. Levy, *Biochim. Biophys. Acta*, 1954, **15**, 589; R. A. Turner and G. Schmerzler, *ibid.*, 1954, **13**, 553; M. Dautravaux and G. Biserte, *Compt. rend.*, 1955, **240**, 1153; S. W. Fox, T. L. Hurst, J. F. Griffith, and O. Underwood, *J. Amer. Chem. Soc.*, 1955, **77**, 3119.
[66] R. A. Boissonnas, *Nature*, 1953, **171**, 304.
[67] A. R. Thompson, *Biochim. Biophys. Acta*, 1954, **15**, 299.
[68] S. Akabori, K. Ohno, and K. Narita, *Bull. Chem. Soc. Japan*, 1952, **25**, 214; K. Ohno, *J. Biochem. Japan*, 1953, **40**, 621.
[69] K. Schlögl and E. Wawersich, *Naturwiss.*, 1954, **41**, 38; K. Schlögl, F. Wesselly, and E. Wawersich, *Monatsh.*, 1954, **85**, 957.
[70] R. W. Chambers and F. H. Carpenter, *J. Amer. Chem. Soc.*, 1955, **77**, 1527.

and pyridine at 150° into ketones which are liberated on complete hydrolysis of the peptide bonds.[71]



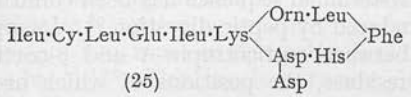
Of the methods of stepwise degradation for the determination of sequence the route *via* an amino-alcohol is probably the most promising. It is generally agreed [72] that lithium borohydride is preferable to lithium aluminium hydride for reduction of the free carboxyl group, and it has been shown that isomerisation of the β-hydroxy-amide (22) by acid or acid chloride to the β-amino-ester (23) is effected in 85—90% yield.[73] Reduction of the ester with lithium borohydride gives the amino-alcohol in 85—90% yield and a new β-hydroxy-amide (24) which can then be treated with phosphorus oxychloride, so that the series of processes can be repeated.



Biologically Active Peptides.—New methods involving chromatography on charcoal and zone electrophoresis have been described [74] for the purification of bacitracin A, which is now believed to have the empirical formula $C_{66}H_{103}O_{16}N_{17}S$ and to contain three *iso*leucine residues.[75, 76] The presence of *alloiso*leucine is confirmed,[76] but there is general agreement that the amino-acid sequence of Porath [77] is incorrect and that the sequence (25) more correctly represents the structure.[78, 79, 80, 81, 82] The possibilities cannot be ruled out that interaction occurs also between phenylalanine and the terminal *iso*leucine residue [79] and that the two aspartic acid residues are joined in an unbranched chain.[81] It appears probable that, of these two residues, the

[71] R. A. Turner and G. Schmerzler, *J. Amer. Chem. Soc.*, 1954, **76**, 949.
[72] W. Grassmann, H. Hörmann, and H. Endres, *Chem. Ber.*, 1953, **86**, 1477; 19
88, 102; M. Justisz, D. M. Meyer, and L. Penasse, *Bull. Soc. chim. France*, 1954, 10
J. C. Crawhall and D. F. Elliott, *Biochem. J.*, 1955, **61**, 264.
[73] J. L. Bailey, *ibid.*, 1955, **60**, 173.
[74] J. Porath, *Acta Chem. Scand.*, 1954, **8**, 1813.
[75] L. C. Craig, W. Hausmann, and J. R. Weisiger, *J. Amer. Chem. Soc.*, 1954,
2839.
[76] W. Hausmann, J. R. Weisiger, and L. C. Craig, *ibid.*, 1955, **77**, 721.
[77] J. Porath, *Nature*, 1953, **172**, 871.
[78] A. I. M. Lockhart, G. G. F. Newton, and E. P. Abraham, *ibid.*, 1954, **173**, 53
[79] A. I. M. Lockhart and E. P. Abraham, *Biochem. J.*, 1954, **58**, 633.
[80] *Idem*, *J. Amer. Chem. Soc.*, 1954, **76**, 2839.
[81] W. Hausmann, J R. Weisiger, and L. C. Craig, *ibid.*, 1955, **77**, 723.
[82] J. R. Weisiger, W. Hausmann, and L. C. Craig, *ibid.*, p. 731.

attached to lysine is L-aspartic acid and is joined to the ε-amino-group.[83] *alloiso*Leucine is believed to be next to the latent cysteine residue, which, as suggested by Newton and Abraham,[84] is combined to form a thiazoline ring. This formulation is in agreement with the ultraviolet absorption of the



(25)

peptide and the fact that the yield of the dinitrophenyl derivative of *iso*leucine is increased by previous oxidation of the sulphur-containing residue with performic acid.[75]

Oxytocin and vasopressin continue to receive considerable attention and, following the full description of the first synthesis of oxytocin,[85] a new synthesis of the hormone has been described [86] which makes use of the same final stage as was used by Du Vigneaud's school. The unusual fission with bromine water of a peptide bond in performic acid-oxidised oxytocin, discussed in a previous Report,[87] has been shown not to be connected necessarily with the bromination of tyrosine, since this can be achieved without cleavage of the peptide bond by using glacial acetic acid or dilute hydrobromic acid as solvent.[88] Nevertheless, prevention of bromination by conversion of the phenolic group of tyrosine into the dinitrophenyl ether renders the peptide bond stable towards bromine water.

Full details of the determination of the sequence of amino-acids in arginine-vasopressin have now been published,[89] and, by similar methods to those used for lysine-vasopressin, a synthetic polypeptide has been obtained having the same relative pressor, antidiuretic, and avian vasopressor activities as arginine-vasopressin.[90]

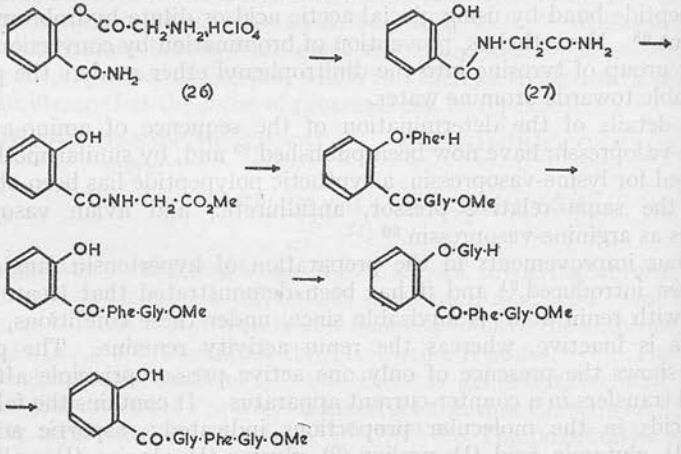
Various improvements in the preparation of hypertensin (angiotonin) have been introduced,[91] and it has been demonstrated that treatment of plasma with renin at 0° is advisable since, under these conditions, hypertensinase is inactive, whereas the renin activity remains. The purified peptide shows the presence of only one active pressor principle after one hundred transfers in a counter-current apparatus. It contains the following amino-acids in the molecular proportions indicated: aspartic acid (2), serine (1), glutamic acid (1), proline (2), glycine (1), alanine (1), valine (1), *iso*leucine (1), tyrosine (1), phenylalanine (1), leucine (2), histidine (2), lysine (1), and arginine (2). Fission of the peptide by hydrazine indicates that either leucine or *iso*leucine is the *C*-terminal residue, and examination of the

[83] A. I. M. Lockhart and E. P. Abraham, *Biochem. J.*, 1954, **58**, xlvii.
[84] G. G. F. Newton and E. P. Abraham, *ibid.*, 1953, **53**, 604.
[85] V. Du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, and P. G. Katsoyannis, *J. Amer. Chem. Soc.*, 1954, **76**, 3115.
[86] R. A. Boissonnas, S. Guttmann, P. A. Jaquenoud, and J. P. Waller, *Helv. Chim. Acta*, 1955, **38**, 1491.
[87] *Ann. Reports*, 1953, **50**, 269.
[88] C. Ressler and V. Du Vigneaud, *J. Biol. Chem.*, 1954, **211**, 809.
[89] E. A. Popenoe and V. Du Vigneaud, *ibid.*, 1954, **206**, 353; R. Archer and J. Chauvet, *Biochim. Biophys. Acta*, 1954, **14**, 421.
[90] V. Du Vigneaud, D. T. Gish, and P. G. Katsoyannis, *J. Amer. Chem. Soc.*, 1954, **76**, 4751.
[91] L. C. Clark, C. Winkler, F. Gollan, and R. P. Fox, *J. Biol. Chem.*, 1954, **206**, 717; A. A. Green and F. M. Bumpus, *ibid.*, 1954, **210**, 281.

dinitrophenyl derivative shows aspartic acid as the only *N*-terminal group. The last fact supports the claim for the homogeneity of the preparation.[92]

The complete sequence of amino-acids in β-corticotropin has been determined.[93] The order of most of the residues is also known for corticotropin-A,[94] and the *N*-terminal sequence has been confirmed by synthesis of the pentapeptide produced by peptic digestion.[95] It appears that the only possible difference between corticotropin-A and β-corticotropin is in the sequence of seven residues, the positions of which are uncertain in the former peptide; also corticotropin-A probably contains no amide groups.[96] α-Corticotropin has the same terminal tripeptide sequence as corticotropin-A but differs from it in amino-acid content and partition behaviour.[97]

Synthetic Peptides.—An excellent survey of the methods of peptide synthesis has appeared [98] and only more recent developments will be discussed. A thorough examination of the use of *N*-substituted amides of phosphorous and phosphoric acids has been made.[99] An unusual rearrangement of compounds such as the perchlorate of *O*-glycylsalicylamide (26) to salicylglycine amide (27) affords a route for the synthesis of peptides as indicated.[100]



In addition to the use of the benzyl residue for protecting the amino group during peptide synthesis,[101] the triphenylmethyl residue has been recommended [102] since it is readily removed with aqueous acetic acid.

[92] F. M. Bumpus, A. A. Green, and I. H. Page, *J. Biol. Chem.*, 1954, **210**, 287.
[93] K. S. Howard, R. G. Shepherd, E. A. Eigner, D. S. Davies, and P. H. Bell, *Amer. Chem. Soc.*, 1955, **77**, 3419.
[94] W. F. White and W. A. Landmann, *ibid.*, p. 771.
[95] K. Hofmann and A. Jöhl, *ibid.*, p. 2914.
[96] W. F. White and W. A. Landmann, *ibid.*, p. 1711.
[97] J. I. Harris and C. H. Li, *J. Biol. Chem.*, 1955, **213**, 499.
[98] T. Wieland, *Angew. Chem.*, 1954, **66**, 507.
[99] S. Goldschmidt and F. Obermeier, *Annalen*, 1954, **588**, 24.
[100] M. Brenner, J. P. Zimmermann, J. Wehrmüller, P. Quitt, and I. Photaki, *Experientia*, 1955, **11**, 397.
[101] L. Velluz, J. Anatol, and G. Amiard, *Bull. Soc. chim. France*, 1954, 1449; Velluz, G. Amiard, and R. Heymès, *ibid.*, 1955, 201.
[102] G. Amiard, R. Heymès, and L. Velluz, *ibid.*, p. 191.

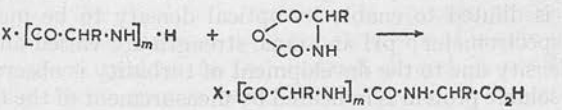
further method of somewhat limited application is the use of the benzoyl-L-phenylalanyl residue which is removed by the action of chymotrypsin.[103] It has been found that reduction of the benzyloxyimino-group, first suggested by Weaver and Hartung,[104] in ammoniacal solution favours the formation of peptides and avoids the production of diketopiperazines.[105] The *N*-trifluoroacetyl residue as a protecting group [106] has found further application,[107] and an important new route to the synthesis of trifluoroacetamido-acids has been introduced.[108] This involves transacylation between the amino-acid and ethyl trifluorothiolacetate:

$$CF_3{\cdot}CO{\cdot}SEt + NH_2{\cdot}CH_2{\cdot}CO_2H \longrightarrow EtSH + CF_3{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO_2H$$

The trifluoroacetylglycine was converted by the phenyl thiol ester method of Wieland and his co-workers [109] into a dipeptide, and the protective group was readily removed at a pH above 10. No racemisation was observed during the above processes. It has been reported that *p*-nitrophenyl esters are more satisfactory for peptide synthesis.[110] A new method of forming the peptide bond has been developed simultaneously in two laboratories, using di*cyclo*hexylcarbodi-imide.[111] The method is not sensitive to moisture, in contrast to those using mixed anhydrides, and the di*cyclo*hexylurea which is formed as a by-product is readily removed.

$$R{\cdot}CO_2H + H_2N{\cdot}R' + C_6H_{11}{\cdot}N{:}C{:}N{\cdot}C_6H_{11} \longrightarrow R{\cdot}CO{\cdot}NH{\cdot}R' + C_6H_{11}{\cdot}NH{\cdot}CO{\cdot}NH{\cdot}C_6H_{11}$$

Space does not permit discussion of all the syntheses of polyamino-acids which have been described in the past two years. The method using the "Leuchs anhydride" as monomer has been applied to the synthesis of polymers containing various side chains such as poly-(*S*-allylcysteine),[112] polytryptophan,[113] and poly-*p*-aminophenyl-DL-alanine.[114] By initiation of the polymerisation with polylysine, cross-linked polymers have been obtained.[115] Termination of the polymerisation of "Leuchs anhydrides" may take place as shown, since it has been found that ureido end-groups are present: [116]



A polyglutamic acid has also been obtained by this method using γ-benzyl L-glutamate as monomer, the benzyl groups being removed with phosphon-

[103] R. W. Holley, *J. Amer. Chem. Soc.*, 1955, **77**, 2552.
[104] W. E. Weaver and W. H. Hartung, *J. Org. Chem.*, 1950, **15**, 741.
[105] W. H. Hartung, D. N. Kramer, and G. P. Hager, *J. Amer. Chem. Soc.*, 1954, **76**, 2261.
[106] F. Weygand and E. Csendes, *Angew. Chem.*, 1952, **64**, 136.
[107] F. Weygand and E. Leising, *Chem. Ber.*, 1954, **87**, 248; F. Weygand and M. Reiher, *ibid.*, 1955, **88**, 26.
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ium iodide.[117] It has been shown that removal of ester groups from meth poly-α-glutamate with 0·5N-sodium hydroxide in the presence of fresh precipitated copper hydroxide produces no racemisation.[118] Synthesis a γ-linked polyglutamic acid *via* a dipeptide ester gave a product whic resembled a natural bacterial polyglutamic acid and differed from pol α-glutamic acid in solubility, in giving a strongly positive ninhydrin reactio in titration constant, and in infrared spectrum.[119] A mixed αγ-polyglutam acid has also been obtained by polymerisation of an αγ-dipeptide este followed by hydrolysis of ester residues.[120] Among other studies of pepti ester polymerisation, it is important to notice that di- and tri-peptide este polymerise more readily than higher members of the series.[121] It was al shown that azides polymerise in aqueous solution to give polyamino-aci of high molecular weight. On the other hand, the azide of triglycine h been converted into a cyclic peptide [122] which it is now agreed [123] is a cyc hexaglycine identical with a product obtained by the " Leuchs anhydride method.[124] Other monomers which have been used include *N*-phenylthi carbonyl derivatives [125] and acyl chlorides,[126] the latter being particular useful for the polymerisation of β-amino-acids. Synthetic polypeptid containing more than one type of functional group have been prepared b the " Leuchs anhydride " method,[127] and also by ester condensation.[12 Finally, an interesting approach to the production of polyamino-acids exemplified by the formation of a polyphenylalanine by introducing fun tional groups into a styrene polymer.[129]

Isolation and Purification of Proteins.—It has been found [130] that, careful attention to conditions such as pH, some of the older protein pr cipitants such as metallic tungstates, sulphosalicylic acid, and metaph phates can be used with advantage for the fractionation of serum protei On the other hand, precipitation of serum proteins by acids is complete on on heating.[131] A very simple method has been described for carrying o preliminary experiments as a guide in devising suitable techniques for t resolution of mixtures of proteins : [132] the solution containing the mixtu of proteins is diluted to enable its optical density to be measured in t ultraviolet spectrometer; pH and ionic strength are varied and the increa in optical density due to the development of turbidity is observed; then t decrease in soluble protein is indicated by measurement of the optical densi

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of the supernatant liquid after centrifugation. It has been pointed out that unexpected results may be obtained in the solubility test for homogeneity owing to what appears to be an isomorphic transformation in the solid phase.[133] Fractional separation from concentrated aqueous solution has been used for the purification of clupein, and the purified material had only proline as end-group.[134] In a development of previous work, blood-clotting factors and serum proteins have been purified by chromatography on diatomaceous earths, different commercial specimens of which vary in adsorptive capacity.[135]

The two chains of performic acid-oxidised insulin have been separated and isolated with a recovery of 95% by counter-current distribution.[136] Although the phenylalanyl chain was not separated from unoxidised insulin, the recovery is much better than that originally obtained by solvent precipitation. The isolated peptides may also be purified by partition chromatography.[137] Chromatographic separation of the A and the B chain of reduced insulin has also been reported.[138] By careful choice of a critical pair of phases, a direct separation of γ-globulin and albumin can be obtained.[139] An outstanding success has been achieved by using partition chromatography, in that γ-globulin from immune rabbits has been partially separated into inert globulin and antibody.[140]

The separation of neutral proteins by chromatography on IRC–50 resin is well established. Closely related carbon monoxide hæmoglobins have been separated by this means and it has been shown that the eluted proteins are unaltered and can be readily crystallised.[141] Ion-exchange chromatography of acidic proteins presents some difficulties since IRC–50 adsorbs these too strongly and basic polystyrene resins such as Dowex-50 are unsuitable because of the physical form of the polymer. However, kieselguhr coated with a cross-linked polystyrene resin has been used with success.[142]

Electrophoresis continues to be used very largely for the separation of proteins, and the subject has been reviewed by Tiselius.[143]

Structure of Proteins.—As indicated in a previous Report,[87] the two outstanding problems concerning the structure of insulin involve the positions of the amide and the disulphide residue. It appears possible, however, from a study of optical rotations, that the structure of insulin itself may be different from those present in the derived A and B peptides.[144] The positions of the amide groups have been determined by estimating the relative ionophoretic mobilities and amide contents of peptides obtained from enzymic digests of A and B fractions of oxidised insulin.[145] The difficulty introduced by the occurrence of disulphide-interchange reactions[146]

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has been overcome by digesting insulin with chymotrypsin and oxidising the peptides formed, and by this means the disulphide linkages have been located.[147] Disulphide cross-linkages have been studied also in connection with other proteins. For instance, it has been shown that the reduction of disulphide groups in wheat gluten is accompanied by loss of elasticity and cohesion.[148] However, in wool, the susceptibility of cystine linkages to reduction is governed by their accessibility to the reagent.[149] A disulphide dimer of human mercaptalbumen has been produced by oxidation with iodine of the mercury dimer.[150] Other types of interchain links are present in collagen, and a direct correlation has been found between intermolecular cohesion in the protein and its hydroxyproline content.[151] It is believed that linkages occur between hydroxyl and keto-imide groups.[152] There has been considerable disagreement as to the nature of the units in the collagen molecule, but it is now claimed that they are rigid, rod-shaped particles of fairly uniform size.[153]

Several studies have been made concerning the nature of the linkages in conjugated proteins. For instance, pepsin and ovalbumen have been degraded to peptides to which the original phosphate residue of the protein is still attached by esterification.[154] Similarly, fibrinogen has yielded a peptide containing tyrosine *O*-sulphate [155] and cytochrome *c* has been degraded by peptic digestion to a hæmopeptide, the configuration of which has been discussed.[156] The present state of knowledge of the configuration of a number of proteins has been reviewed.[157]

G. R. B.

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674. *The Structure of the Extracellular Polysaccharide of* Aerobacter aerogenes A3 (S1) (*Klebsiella Type* 54).

By G. O. Aspinall, R. S. P. Jamieson, and J. F. Wilkinson.

Preliminary studies have shown that the extracellular slime polysaccharide of *Aerobacter aerogenes* A3 (S1) possesses a highly branched molecular structure containing residues of D-glucose, L-fucose, and glucuronic acid. Cellobiose has been identified amongst the products of partial acid hydrolysis. A number of methylated sugars, including 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, 2 : 3-di-*O*-methyl-D-glucose, 3 : 5-di-*O*-methyl-L-fucose, and 2-*O*-methyl-L-fucose, have been characterised from the products of hydrolysis of the methylated polysaccharide.

In a previous study [1] it was shown that the extracellular slime polysaccharide of *Aerobacter aerogenes* A3 (S1) (Klebsiella Type 54), isolated as the free acid, was composed of three sugar residues, D-glucose (46%), L-fucose (10%), and an unidentified uronic acid (27%), together with traces of galactose (*ca.* 2%). It was also shown that the composition of the polysaccharide was independent of the carbon source, when the bacterium was grown in the presence of a variety of carbohydrates as the sole carbon and energy source. A start has now been made in the determination of the molecular structure.

The nature of the uronic acid residue was established as glucuronic acid by the following observations: (*a*) glucurone was detected by paper chromatography when the polysaccharide was hydrolysed with 4N-hydrochloric acid; (*b*) glucose was the only hexose detected in significant amount when the polysaccharide was hydrolysed after reduction of the acidic residues (as methyl esters) with potassium borohydride; and (*c*) estimation of the glucose and fucose formed on hydrolysis of the polysaccharide, before and after reduction of the uronic acid to hexose residues, showed that the ratio (by weight) of glucose to fucose increased from 4·0 : 1 to 5·8 : 1. Although the value obtained for the ratio of glucose to fucose given on direct hydrolysis of the polysaccharide was slightly different from that obtained in the previous investigation,[1] it is clear that treatment of the uronic acid residues (as methyl ester) with potassium borohydride has resulted in the formation of further glucose residues; it is probable, however, that reduction with this reagent (cf. sodium borohydride [2]) was not complete.

Chromatographic examination of the products of graded hydrolysis of the polysaccharide showed that some of the glucose was released readily and that shortly afterwards cellobiose could also be detected. On prolonged heating, fucose, traces of galactose, and more glucose were formed. On a larger scale the products of partial acid hydrolysis were separated chromatographically, and cellobiose and D-glucose were identified as crystalline derivatives. The partially degraded polysaccharide yielded L-fucose and more D-glucose on more vigorous hydrolysis, together with a mixture of acidic oligosaccharides, all of which gave glucose, fucose, and glucurone after drastic hydrolysis.

The polysaccharide was methylated by Fear and Menzies's method.[3] The methylated sugars obtained on hydrolysis of the methylated polysaccharide were fractionated chromatographically on cellulose,[4] and the following were characterised as crystalline derivatives: 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, 2 : 3-di-*O*-methyl-D-glucose, 3 : 5-di-*O*-methyl-L-fucose, and 2-*O*-methyl-L-fucose. Evidence was also obtained for the presence of a trace of 2 : 3 : 4-tri-*O*-methyl-D-glucose, a mixture of tri-*O*-methyl-D-glucoses (including the 2 : 3 : 6-isomer, as shown by the downward change in rotation in methanolic hydrogen chloride), a mixture of 2 : 6- and 3 : 6-di-*O*-methyl-D-glucoses, and a mixture of mono-*O*-methylglucoses (including the 2- and the 3-methyl ether). In addition, a complex acidic fraction was obtained from this hydrolysis, but attempts to isolate individual components failed. After hydrolysis under drastic conditions, chromatography showed tri-, di-, and mono-*O*-methyl-uronic acids to be present; furthermore, after reduction of the acidic

fraction with lithium aluminium hydride, hydrolysis yielded sugars travelling on the chromatogram at the same rate as tri-, di-, and mono-*O*-methylglucoses.

The previously unknown sugar 3 : 5-di-*O*-methyl-L-fucose was obtained crystalline, and its structure was established from the following observations : (*a*) demethylation showed the sugar to be a derivative of fucose ; (*b*) the derived di-*O*-methyl-L-fuconolactone underwent hydrolysis at a rate characteristic of γ-lactones, and the sign of the optical rotation, according to Hudson's lactone rule,[5] was only consistent with that of a 1 : 4-lactone ; (*c*) the consumption of 1 mol. of periodate by the sugar suggested that the sugar was the 3 : 5-dimethyl ether rather than the 2 : 5-dimethyl ether of L-fucose, and the absence of a methoxyl group at $C_{(2)}$ was confirmed, as the derived di-*O*-methyl-L-fuconamide gave a positive Weerman test.

It is now clear that the molecule is highly branched and that the following sugar residues are definitely present :

$$\text{Gp 1}\cdots, \quad \cdots\cdot 4\ \text{Gp 1}\cdots, \quad \cdots\cdot 2\ \text{Fuc}f\ 1\cdots, \quad \cdots\cdot 4(\text{or 5})\ \text{Fuc 1}\cdots$$

$$\vdots\ 3$$

(Gp = D-glucopyranose, Fuc*f* = L-fucofuranose)

The isolation of cellobiose on graded hydrolysis of the polysaccharide shows that adjacent β-1 : 4-linked D-glucopyranose residues are present. It is not clear, however, which of the dimethyl and monoethyl ethers of D-glucose have structural significance as arising from branching points and which have arisen from incomplete methylation of the polysaccharide and/or demethylation during hydrolysis. On the other hand, it is certain that some of the L-fucose residues are branching points in the molecule as 2-*O*-methyl-L-fucose cannot have been formed from the incomplete methylation of 3 : 5-di-*O*-methyl-L-fucose, the only other methyl-L-fucose isolated. The release of 1 mole of formic acid per 3 sugar residues on periodate oxidation of the polysaccharide suggests the presence of a high proportion of non-reducing terminal groups and/or 1 : 6-linked D-glucose residues. In view of the small amount of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose isolated on hydrolysis of the methylated polysaccharide and in the absence of more than traces of 2 : 3 : 4-tri-*O*-methyl-D-glucose, it seems probable that some of the glucuronic acid residues may occupy terminal positions in the molecule.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the solvent systems : (*A*) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3; v/v; top layer); (*B*) butan-1-ol–ethanol–water (4 : 1 : 5; v/v; top layer); (*C*) butan-1-ol–acetic acid–water (4 : 1 : 5; v/v; top layer); (*D*) butan-1-ol saturated with 5% aqueous formic acid.

Isolation and Examination of the Extracellular Polysaccharide.—The organism was grown in a buffered medium (pH 7·3) containing D-glucose as sole carbon source, and the extracellular polysaccharide was isolated as the free acid as described by Wilkinson, Dudman, and Aspinall.[1] The polysaccharide had an equivalent of 683 (by titration), corresponding to 25·8% uronic anhydride (compare 29·0% uronic anhydride previously found). The uronic anhydride content was also determined by Kaye and Kent's method [6] after the polysaccharide had been converted into the corresponding methyl ester by heating with methanolic hydrogen chloride, a value of 25·5% being obtained. Direct reaction of the polysaccharide with the same reagents showed that ester and lactone groups were absent and no methoxyl content was detected. When the polysaccharide was hydrolysed and the hydrolysate was examined chromatographically, the three sugars described previously (glucose, fucose, and galactose) were detected, but after hydrolysis with 4N-hydrochloric acid glucurone was also found.

Hydrolysis of the Polysaccharide after Reduction with Potassium Borohydride.—The polysaccharide (18·3 mg.) was heated in a sealed tube with 98% formic acid (2 c.c.) at 100° for 24 hr., the formic acid was removed *in vacuo*, L-rhamnose (5·45 mg.) was added, and the mixture was refluxed with methanolic 1% hydrogen chloride (5 c.c.) for 4 hr. After neutralisation with silver carbonate the product was dissolved in water (2 c.c.), and the solution was added dropwise to a solution of potassium borohydride (20 mg.) in water (2 c.c.). After 20 min. the excess of borohydride was destroyed by dilute acetic acid, and the solution was de-ionised

with Amberlite resins IR-120(H) and IR-4B(OH). The reduction product was hydrolysed with N-hydrochloric acid (4 c.c.) at 100° for 4 hr. and neutralised, and the ratios of the sugars obtained were determined. A second sample of polysaccharide was treated similarly except that the reduction with potassium borohydride was omitted.

The results showed that the ratio of glucose to fucose was 5·8 : 1 in the former case and 4·0 : 1 when the reduction was omitted. The ratios of fucose to the reference sugar, rhamnose, were the same in both cases.

Graded Hydrolysis of the Polysaccharide.—Chromatographic examination of the products of the hydrolysis of the polysaccharide with hot 0·5N-hydrochloric acid, showed that glucose was released after 5 min. and that after 30 min. a disaccharide travelling on the chromatogram at the same rate as cellobiose was observed. On further heating fucose and traces of galactose were also released.

The polysaccharide (2 g.) was heated at 100° with 0·5N-sulphuric acid (100 c.c.) for 30 min. The cooled solution was neutralised with barium carbonate, and the filtrate was reduced in volume to 10 c.c. and poured into acetone (20 c.c.). The precipitate was redissolved in water, and the solution de-ionised with Amberlite resin IR-120(H) and taken to dryness to give fraction *X* (0·84 g.). The supernatant liquid remaining after the acetone precipitation was taken to dryness to give fraction *Y* (0·70 g.). Most (0·67 g.) of this fraction was separated on filter sheets by using solvent *A* to give small fractions containing sugars travelling on the chromatogram at the same rate as glucose, galactose, and cellobiose, whilst most of the material, fraction *Y*(i) (0·53 g.), remained at the starting line. D-Glucose and cellobiose were identified by conversion into 1 : 2 : 3 : 4 : 6-penta-*O*-acetyl-β-D-glucose, m. p. 126—128° and mixed m. p. 127—130°, and cellobiose α-octa-acetate, m. p. and mixed m. p. 220—222°, respectively.

Chromatographic examination of fractions *X* and *Y*(i) in solvent *C* showed them to contain similar mixtures of acidic oligosaccharides. It was not possible completely to separate the components, but all the fractions examined yielded on further hydrolysis D-glucose, L-fucose (identified as the toluene-*p*-sulphonylhydrazone, m. p. and mixed m. p. 167—170°), galactose (trace), and glucurone. When the acidic fractions were reduced by treatment of the corresponding methyl ester methyl glycosides with potassium borohydride, the hydrolysis products contained glucose, fucose, and galactose (trace).

Methylation of the Polysaccharide.—The polysaccharide (5 g.) was converted into its thallium derivative which was heated with methyl iodide, and the product was further methylated by three treatments with thallous ethoxide and methyl iodide; this gave a methylated polysaccharide (3·7 g.) (Found : OMe, 39·6%), whose methoxyl content could not be raised on further treatments with thallous ethoxide and methyl iodide.

Hydrolysis of the Methylated Polysaccharide and Separation of the Methylated Sugars.—The methylated polysaccharide (3·2 g.) was hydrolysed successively with methanolic 1% hydrogen chloride (200 c.c.) for 17 hr. (constant rotation) and with hydrochloric acid (200 c.c.; 0·5N) for 10·5 hr. (constant rotation). The solution was neutralised with silver carbonate, the silver ions were removed with hydrogen sulphide, and the acidic components were converted into barium salts by treatment with barium carbonate. The solution was evaporated to a dark brown glass (2·8 g.).

The hydrolysate was dissolved in the minimum quantity of water, and the resulting syrup was allowed to soak into the top of a column (60 × 3 cm.) of cellulose. Elution of the column with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later 1 : 1), saturated with water, butan-1-ol partly saturated with water, and water gave nine fractions.

Fraction 1. The syrup (8 mg.) had $[\alpha]_D^{20}$ +87° (*c*, 0·4 in H_2O) and travelled on the chromatogram at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose. The sugar was identified as this compound by conversion into the aniline derivative, m. p. 123—124° and mixed m. p. 125—127° (with an authentic sample, m. p. 128—129°). In a separate series of experiments 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose crystallised, had m. p. and mixed m. p. 84—86°, and its *X*-ray powder photograph (courtesy of Dr. C. A. Beevers) was identical with that of an authentic specimen.

Fraction 2. The syrup (5 mg.) had $[\alpha]_D^{20}$ +63°, travelled on the chromatogram at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucose (R_G 0·87), and gave glucose on demethylation (Found : OMe, 41·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction 3. Chromatographic examination of the syrup (122 mg.) showed the presence of two components travelling at the rates of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methyl-D-glucose (R_G 0·81—0·83) (Found : OMe, 41·6. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Demethylation gave only glucose and the presence of 2 : 3 : 6-tri-*O*-methyl-D-glucose in the mixture was shown

by the fall in rotation in methanolic 2% hydrogen chloride at room temperature {$[\alpha]_D^{20}$ $+73° \longrightarrow +35°$ (26 hr., const.; *c*, 1·8)}.

Fraction 4. The syrup (76 mg.) was chromatographically homogeneous (R_G 0·79—0·80 in solvent *B*), gave a green colour with aniline oxalate, had $[\alpha]_D^{20}$ $-72° \longrightarrow -67°$ (24 hr., const.; *c*, 1·5 in H_2O), and yielded fucose on demethylation with hydriodic acid (Found: OMe, 32·2. Calc. for a 6-deoxy-di-*O*-methylhexose, $C_8H_{16}O_5$: OMe, 32·3%). The derived 6-deoxy-di-*O*-methylhexonolactone had $[\alpha]_D^{20}$ $+66° \longrightarrow +51°$ (7 days, const.; *c*, 0·66 in H_2O) and was converted into a 6-deoxy-di-*O*-methylhexonamide, which did not crystallise but yielded hydrazodicarbonamide, m. p. and mixed m. p. 252—254°, when treated with sodium hypochlorite. The original syrup consumed 1·15 moles of periodate per $C_8H_{16}O_5$ unit.

In separate experiments 3 : 5-*di*-O-*methyl*-L-*fucose* was obtained crystalline; it had m. p. 118—121°, $[\alpha]_D^{20}$ $-100° \longrightarrow -69°$ (24 hr., const.; *c*, 0·42 in H_2O) (Found: OMe, 32·2. $C_8H_{16}O_5$ requires OMe, 32·3%).

Fraction 5. The syrup (45 mg.) travelled on the chromatogram at the same rate as 2 : 3-di-*O*-methyl-D-glucose and gave glucose on demethylation. The sugar had $[\alpha]_D^{20}$ $+64°$ (equil.; *c*, 0·52 in H_2O) (Found: OMe, 29·7. Calc. for $C_8H_{16}O_6$: OMe, 29·7%), and was identified by conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-glucosylamine, m. p. and mixed m. p. 129—131°.

Fraction 6. The syrup (164 mg.) was chromatographically homogeneous (R_G 0·54 in solvent *B*) but the methoxyl content (23·4%) suggested the presence of two components. Fractionation gave an acetone-insoluble fraction 6*a* (43 mg.) and an acetone-soluble fraction 6*b* (121 mg.). Fraction 6*a* was obtained crystalline and yielded fucose on demethylation. The sugar had m. p. 142—145° and mixed m. p. (with 2-*O*-methyl-L-fucose, m. p. 147—149°) 144—148°, $[\alpha]_D^{20}$ $-71°$ (initial) $\longrightarrow -81°$ (24 hr., const.; *c*, 0·33 in H_2O) (Found: OMe, 17·5. Calc. for $C_7H_{14}O_5$: OMe, 17·4%). Fraction 6*b* yielded glucose on demethylation, travelled on the chromatogram at the same rate as 2 : 6- and/or 3 : 6-di-*O*-methyl-D-glucose, and had $[\alpha]_D$ $+60°$ (equil.; *c*, 2·0 in H_2O). Periodate oxidation of the derived methyl glycosides[7] showed that 83% of the fraction was 2 : 6-di-*O*-methyl-D-glucose. The periodate-oxidised methyl glycosides were hydrolysed, and chromatographic examination showed the presence of 3 : 6-di-*O*-methyl-D-glucose.

Fraction 7. The syrup (97 mg.) travelled on the chromatogram at the same rate as 2 : 6- and/or 3 : 6-di-*O*-methyl-D-glucose in solvent *B* but another component was detected by chromatography in solvent *A*. Fractionation of a portion of the syrup in solvent *A* yielded fraction 7*a* (40 mg.) and fraction 7*b* (4 mg.). Periodate oxidation of the derived methyl glycosides showed fraction 7*a* to contain 80% of 2 : 6-di-*O*-methyl-D-glucose, and hydrolysis of the periodate-oxidised methyl glycosides gave 3 : 6-di-*O*-methylglucose. Fraction 7*b* had R_G 0·50 in solvent *B* (yellow colour with aniline oxalate) and gave glucose on demethylation.

Fraction 8. Chromatographic examination of the syrup (190 mg.) in solvent *A* indicated only one component (R_G 0·20—0·22), but on examination in solvent *B* three substances were shown to be present, one of them travelling at the same rate as L-fucose. A portion of the syrup was oxidised by periodate according to Lemieux and Bauer's[8] procedure; chromatographic examination showed the products to be similar to those formed on oxidation of 2- and 3-*O*-methyl-D-glucose. The two faster components were separated from fucose on filter sheets by using solvent *A* to give fraction 8*a* (64 mg.) which yielded glucose on demethylation (Found: OMe, 16·0. Calc. for $C_7H_{14}O_6$: OMe, 16·0%).

Fraction 9. This fraction (1·30 g.) obtained on elution of the cellulose column with water was present as the barium salt and had equivalent wt. 223. Drastic hydrolysis with 2N-sulphuric acid followed by chromatographic examination in solvent *D* showed that three acidic components, R_G 0·84, 0·58, 0·44 (cherry-red coloration with aniline oxalate), and some neutral sugars were produced. A portion was converted into the methyl ester methyl glycoside, which was reduced with lithium aluminium hydride; hydrolysis of the product yielded sugars travelling on the chromatogram (solvent *B*) at the same rate as tri-, di-, and mono-methylglucoses, together with 3 : 5-di-*O*-methylfucose. An attempt to separate the acidic components by elution from Amberlite resin IRA-400 (acetate form) with increasing concentrations of acetic acid resulted in loss of the sugar acids by adsorption and/or decomposition on the resin.

Periodate Oxidation of the Polysaccharide.—The polysaccharide (154·3 mg.) was dissolved in potassium chloride solution (60 c.c.; 0·56M) and the pH of the solution was adjusted to 6·25. Sodium metaperiodate solution (20 c.c.; 0·20M) was added and the solution was shaken in the dark. Aliquot portions (10 c.c.) were removed from time to time, ethylene glycol (1 c.c.) was added, and the mixture was titrated against 0·108N-sodium hydroxide to pH 6·25 in a

stream of nitrogen. After 160 hr. the formic acid released corresponded to 0·34 mole per 162 g. of polysaccharide.

The polysaccharide (101·6 mg.) was dissolved in water (50 c.c.), and sodium metaperiodate solution (50 c.c.; 0·40M) was added. Estimation of the periodate consumed showed that a constant value of 1·28 mole per 162 g. of polysaccharide was reached after 71 hr. Hydrolysis of a sample of the periodate-oxidised polysaccharide showed the presence of glucose and fucose.

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728. *The Constitution of a Xylan from Norway Spruce* (Picea excelsa).

By G. O. Aspinall and (Miss) Mary E. Carter.

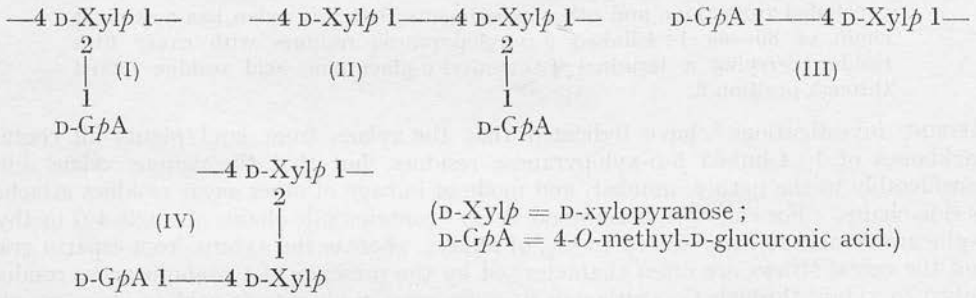
Fractionation of Norway spruce hemicellulose yielded two xylan-rich components. One of these was converted into a fully methylated xylan, hydrolysis of which yielded 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 3-*O*-methyl-D-xylose, and 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose in the approximate molar ratio of 1 : 69 : 8 : 21. It is concluded from these and other experiments that this xylan has a straight chain of 80—85 1 : 4-linked β-D-xylopyranose residues with every fifth residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue linked through position 2.

Recent investigations [1] have indicated that the xylans from land plants all contain backbones of 1 : 4-linked β-D-xylopyranose residues, but that the various xylans differ considerably in the nature, number, and mode of linkage of other sugar residues attached as side-chains. For example, beechwood xylan [2] contains side-chains of single 4-*O*-methyl-D-glucuronic acid residues linked to $C_{(2)}$ of xylose, whereas the xylans from esparto grass and the cereal straws are often characterised by the presence of L-arabofuranose residues linked to xylose through $C_{(3)}$, although in some cases D-glucuronic acid residues are also found. It was of interest, therefore, to extend investigations to the xylans from the coniferous woods or gymnosperms. The isolation of xylobiose and the aldobiouronic acid, 2-*O*-(4-*O*-methyl-D-glucuronosyl)-D-xylose, from the partial acid hydrolysis of black spruce (*Picea nigra*) and Scots pine (*Pinus sylvestris*) [3] suggested that xylans of the type found in beechwood might also be present in these woods. On the other hand, arabinose has been found in the hydrolysates from coniferous woods,[4] and no evidence was available as to whether this sugar was a constituent of a xylose-containing polysaccharide. This paper describes the structure of a xylan isolated from Norway spruce wood (*Picea excelsa*).

The spruce hemicellulose was isolated by extraction of the delignified sawdust with cold aqueous sodium hydroxide; previous experiments had shown that only small yields of alkali-soluble polysaccharides could be obtained by direct extraction of the wood without removal of lignin with chlorous acid. The crude hemicellulose yielded a complex mixture of sugars on hydrolysis, but by careful precipitation from aqueous solution and by precipitation of contaminating polysaccharides as the copper complex two xylan-rich fractions were isolated. Both of these fractions gave, on hydrolysis, acidic oligosaccharides, reduction of whose methyl ester methyl glycosides with potassium borohydride, followed by hydrolysis, yielded 4-*O*-methyl-D-glucose and xylose. As 4-*O*-methyl-D-glucose was not detected on hydrolysis of the xylan fractions, it is clear that this sugar was formed by the reduction of 4-*O*-methyl-D-glucuronic acid, a fairly common constituent of plant polysaccharides.

One of the xylan fractions was converted into its fully methylated derivative, during which process the contaminating polysaccharides containing sugar residues other than xylose and glucuronic acid were lost. Hydrolysis of the methylated xylan gave 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 3-*O*-methyl-D-xylose, together with a mixture of acidic components. The acidic fraction had equivalent weight 378 (Calc. for a glucuronosylxylose tetramethyl ether, 382), but chromatography indicated the presence of at least two components, one of which travelled on the chromatogram at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. The major component of the acidic fraction was identified as 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose in the following way. Reduction of the derived methyl ester methyl glycoside with lithium aluminium hydride

followed by hydrolysis gave 2 : 3 : 4-tri-*O*-methylglucose and 3-*O*-methylxylose, together with a small amount of 2 : 3-di-*O*-methylxylose. A portion of the reduced acidic fraction was remethylated and after hydrolysis yielded two main components, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and 3 : 4-di-*O*-methyl-D-xylose, with traces of 2 : 3 : 4-tri-*O*-methyl- and 3-*O*-methyl-xylose, and 2 : 3 : 4-tri-*O*-methylglucose; no 2 : 3-di-*O*-methylxylose could be detected. It follows from these observations that the 4-*O*-methyl-D-glucuronic acid residues are attached directly to the backbone of D-xylose residues through position 2 as in (I). The chromatographic identification of 2 : 3-di-*O*-methylxylose on hydrolysis of the reduced acidic fraction, and of 2 : 3 : 4-tri-*O*-methyl- and 3-*O*-methyl-xylose on hydrolysis after further methylation suggests that the acidic fraction also contained a trisaccharide derived from the xylan as in (II). The absence of 2 : 3-di-*O*-methylxylose on hydrolysis after remethylation excludes the possibility that the backbone of 1 : 4-linked xylose units might be terminated by a glucuronic acid unit as in (III) or that the side-chains might consist of disaccharide units as in (IV).



The hydrolysis products from the methylated xylan, namely, 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 3-*O*-methyl-D-xylose, and the acidic fraction (calculated as tetra-*O*-methylaldobiouronic acid) were isolated in the approximate molar ratio of 1 : 69 : 8 : 21. A molecular-weight determination by the isothermal-distillation method (by the courtesy of Dr. C. T. Greenwood and Mr. W. N. Broatch) gave a value of 17,000 $\pm$ 500 (degree of polymerisation, 101 $\pm$ 3) for the methylated xylan. This value, taken together with the value of one non-reducing xylose end-group per *ca.* 100 sugar residues, indicates that the backbone of xylose residues is unbranched. It is concluded, therefore, that this xylan has a straight chain of 80—85 1 : 4-linked β-D-xylopyranose residues, with, on the average, every fifth residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue linked through $C_{(2)}$. This xylan is probably only one of many closely related molecular species present in the wood, and it is of interest that no arabinose residues were found. It is possible, therefore, that the arabinose residues present in Norway sprucewood may occur in association with galactose residues, as is the case in the ε-galactan from European [5] and North American [6] larches, and in the arabogalactan from Jeffery pine.[7] However, on present evidence the presence, in spruce, of araboxylans, of the type found in the cereal straws, cannot be excluded.

These results indicate that this Norway spruce xylan resembles closely the hemicellulose A from beechwood,[2] although differing slightly in chain length and in the proportion of 4-*O*-methyl-D-glucuronic acid residues linked as side-chains. Although no detailed structural studies have been carried out on the xylose-containing polysaccharides from aspen,[8] black spruce, and Scots pine [3] woods, it will be recalled that evidence for the presence of xylans of the same general type has been obtained by the isolation of xylobiose and the aldobiouronic acid, 2-*O*-(4-*O*-methyl-D-glucuronosyl)-D-xylose, on partial acid hydrolysis of these woods. Whilst the xylans from different plants [1] exhibit considerable variations in detailed molecular structure, our present knowledge suggests that the xylans from both deciduous and coniferous woods contain the same structural features and differ only in chain length and/or number of uronic acid residues attached as side-chains. However, further investigations of other wood xylans will be necessary before such a generalisation could be regarded as established.

Experimental

Paper chromatography was carried out on Whatman No. 1 filter paper, with the upper layers of the following solvent systems (v/v): (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (B) butan-1-ol–ethanol–water (5 : 1 : 4); (C) butan-1-ol–formic acid–water (500 : 115 : 385). Paper ionophoresis was carried out [9] in borate buffer at pH 10. Optical rotations were observed at room temperature (16—18°).

Extraction and Fractionation of Spruce Hemicellulose.—Extractive-free sawdust was delignified by Wise, Murphy, and D'Addieco's [10] method. In a typical extraction spruce holocellulose (40 g.) was treated with cold 4% aqueous sodium hydroxide under nitrogen for 18 hr. The filtrate was acidified with acetic acid to pH 4 and the crude hemicellulose (3 g.) was precipitated by the addition of two volumes of acetone. Chromatographic examination of the hydrolysate showed xylose, arabinose, mannose, an aldobiouronic acid, and traces of glucose and galactose. Fractionation of the hemicellulose was carried out as follows: Hemicellulose (1 g.) was dissolved in water (100 ml.), acetone (67 ml.) was added, and the solution was acidified to pH 3—4 by sulphuric acid. The resulting precipitate was discarded, sodium carbonate solution was added to bring the supernatant liquor to pH 5—6, and acetone was added until further polysaccharide was precipitated. The precipitated polysaccharide (xylan A; 0·2 g.) was separated and dried.

Spruce holocellulose, exhaustively extracted with 4% aqueous sodium hydroxide, was further extracted with cold 10% aqueous sodium hydroxide under nitrogen for 18 hr., the extract was neutralised with acetic acid, and the precipitate (mainly glucan and mannan) was discarded. Addition of acetone (0·5 vol.) to the supernatant liquor yielded a further precipitate, which gave on hydrolysis xylose, mannose, glucose, an aldobiouronic acid, and a trace of galactose. A xylan-rich fraction was obtained by dissolving the crude hemicellulose (12 g.) in 4% sodium hydroxide solution (1 l.) and adding to the solution glycerol (3 ml.) followed by 0·5M-copper sulphate solution. The pale blue precipitate, which was first formed, was removed at the centrifuge and the addition of copper sulphate was stopped before the dark blue gelatinous precipitate of the xylan complex appeared. The unprecipitated polysaccharide was recovered by neutralisation of the solution with acetic acid followed by precipitation with acetone (*ca.* 0·5 vol.). This procedure was repeated four times until the addition of copper sulphate no longer resulted in the formation of a pale blue precipitate. The polysaccharide (xylan B; 3 g.) isolated from these fractionations gave on hydrolysis xylose and an aldobiouronic acid, together with smaller amounts of mannose, glucose, and galactose.

Examination of Xylan-rich Fractions.—Xylan A had $[\alpha]_D$ −38° (*c*, 0·39 in N-sodium hydroxide) [Found: uronic anhydride (by decarboxylation), 15·0; OMe, 2·7%] and chromatographic examination of the hydrolysate showed the presence of xylose (58%), arabinose (17%), and glucose + galactose (8%). Xylan B had $[\alpha]_D$ −44° (*c*, 0·32 in N-sodium hydroxide) (Found: uronic anhydride, 18·9; OMe, 1·8%) and chromatographic examination of the hydrolysate showed the presence of xylose (63%), mannose (13%), and glucose + galactose (7%).

Xylan A (3 g.) was hydrolysed with N-sulphuric acid (50 ml.) at 100° for 4 hr. The hydrolysate was neutralised with barium carbonate, and barium ions were removed with Amberlite resin IR-120. The resulting solution was poured on to a column of charcoal–Celite, and the monosaccharides were eluted with water. Elution with ethyl methyl ketone–water (1 : 19) yielded a fraction containing aldobiouronic acid, which was converted into the methyl ester methyl glycoside and treated with a solution of potassium borohydride (300 mg.) in water (20 ml.) for 24 hr. Excess of borohydride was destroyed by the addition of acetic acid, and the solution was de-ionised by successive treatments with Amberlite resins IR-120 and IR-4B. The resulting syrup was hydrolysed with 0·8N-hydrochloric acid (15 ml.) for 6 hr. at 100°. Chromatographic examination of the hydrolysate showed the presence of 4-*O*-methylglucose and xylose. The fastest-moving component (56 mg.) was separated on filter sheets, with solvent A, but attempts to characterise the sugar by conversion into the crystalline 4-*O*-methyl-D-glucosazone failed. However, an impure fraction, shown by circular paper chromatography [11] to contain mainly the desired compound, gave an *X*-ray powder photograph with the same lines as those from an authentic sample.

Xylan B (300 mg.) was hydrolysed with N-sulphuric acid (10 ml.) at 100° for 10 hr. The hydrolysate was neutralised with barium carbonate, de-ionised with Amberlite resin IR-120, and concentrated to a syrup. The acidic component (23 mg.) was separated on filter sheets with solvent A, and was converted into the methyl ester methyl glycoside, which was treated with a solution of potassium borohydride (15 mg.) in water (5 ml.) for 24 hr. After destruction

of the excess of borohydride with acetic acid, 2N-hydrochloric acid (5 ml.) was added, and the solution was heated at 100° for 6 hr. After neutralisation and de-ionisation, chromatographic examination of the hydrolysate showed the presence of 4-*O*-methylglucose, xylose, and a trace of glucose.

Methylation of Xylan.—Xylan B (10 g.) was methylated fifteen times with methyl sulphate and sodium hydroxide. The partially methylated polysaccharide, suspended in acetone–water (1 : 1), was stirred with Amberlite resin IR-120 to remove sodium ions, the solution was taken to dryness, and the polysaccharide (4·7 g.) was precipitated from chloroform solution by the addition of light petroleum (b. p. 60—80°). Methylation of the product with methyl iodide and silver oxide gave methylated spruce xylan (4·0 g.) {OMe, 40·3%; $[\alpha]_D$ −43·7° (*c*, 0·25 in $CHCl_3$)}.

Hydrolysis of Methylated Xylan and Separation of Methylated Sugars.—Methylated xylan (3·0 g.) was dissolved in 2N-hydrochloric acid (230 ml.) and kept at room temperature for 17 days. After addition of water (230 ml.), the solution was heated at 100° for 4 hr. and kept at room temperature for 20 hr. This procedure was repeated three times (constant rotation), and a small quantity of insoluble material (*ca.* 0·1 g.) was separated and hydrolysed by successive treatments with boiling methanolic hydrogen chloride and with N-hydrochloric acid at 100°. The combined hydrolysates were neutralised with silver carbonate, the acidic components were converted into barium salts by treatment with barium carbonate, and the solution was concentrated to a syrup (2·9 g.).

The methylated sugars (2·9 g.) were fractionated on cellulose [11] (74 × 3·8 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later, 1 : 1) saturated with water, and butan-1-ol partly saturated with water as eluants, to give five fractions, and a further fraction was obtained by elution of the cellulose with water. Apart from fraction 1 (23 mg.), all other fractions were contaminated with acidic components. Fractions 2 (88 mg.), 4 (0·212 g.), and 6 (0·564 g.) contained only acidic substances and were combined. Portions of fractions 3 (1·510 g.) and 5 (0·188 g.) were each separated on filter sheets with solvent B, and the proportions of acidic and neutral components were determined. The results showed that the following methylated sugars were present in the hydrolysate: tri-*O*-methylxylose [0·023 g., 1·03% (mol.)], di-*O*-methylxylose (1·435 g., 69·2%), mono-*O*-methylxylose (0·161 g., 8·4%), and methylated aldobiouronic acid [0·949 g., 21·3% (calc. as tetramethylaldobiouronic acid)].

Examination of Neutral Sugars.—Fraction 1 crystallised and had m. p. and mixed m. p. (with 2 : 3 : 4-tri-*O*-methyl-D-xylose) 88—91° (Found: OMe, 48·0. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). The neutral component from fraction 3 (Found: OMe, 34·6. Calc. for $C_7H_{14}O_5$: OMe, 34·8%) was identified as 2 : 3-di-*O*-methyl-D-xylose by conversion into the aniline derivative, m. p. and mixed m. p. 122°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 132°. The neutral component from fraction 5 (Found: OMe, 17·9. Calc. for $C_6H_{12}O_5$: OMe, 18·9%) travelled on the chromatogram at the same rate as 2- and/or 3-*O*-methyl-D-xylose, but ionophoretic examination showed the presence of the 3-methyl ether with only traces of the 2-methyl ether.

Examination of the Acidic Fraction.—Chromatographic examination in solvent C of the acidic fractions showed that they all contained two components, one travelling at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. The combined acidic fractions had equiv. 378 (calc. for a tetra-*O*-methylaldobiouronic acid, $C_{15}H_{26}O_{11}$, 382).

The acid (200 mg.) was refluxed with methanolic 1% hydrogen chloride (40 ml.) for 6 hr. The product, after neutralisation, was dissolved in dry ether (65 ml.), lithium aluminium hydride (300 mg.) was added, and the solution was refluxed for 6 hr. Excess of hydride was destroyed by water, and the solution was acidified with sulphuric acid and extracted with chloroform (6 × 50 ml.). The extract was evaporated to a syrup, which was hydrolysed by hot 0·8N-hydrochloric acid for 6 hr. After neutralisation with silver carbonate, part of the hydrolysate was separated on filter sheets with solvent B, to give fractions *a* (61 mg.) and *b* (16 mg.). Fraction *a* contained two components, chromatographically and ionophoretically indistinguishable from 2 : 3 : 4-tri-*O*-methyl-D-glucose (major) and 2 : 3-di-*O*-methyl-D-xylose (minor) severally; the major component was further separated and on demethylation gave only glucose (Found: OMe, 40·0. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Fraction *b* travelled on the chromatogram at the same rate as 2- and/or 3-*O*-methyl-D-xylose, but at a different rate from the 4-methyl ether. Ionophoretic examination showed that only the 3-methyl ether was present.

The acid (375 mg.) was refluxed with methanolic 1% hydrogen chloride (60 ml.) for 6 hr. After neutralisation with silver carbonate and removal of solvent, the methyl ester methyl

glycoside was reduced with lithium aluminium hydride (300 mg.) in boiling methylal (70 ml.) for 2 hr. Excess of hydride was destroyed by water, the methylal layer was separated, and the aqueous layer was extracted with chloroform and ether. The aqueous layer was acidified with dilute sulphuric acid, the sulphate and aluminium ions were removed as precipitates on addition of barium hydroxide, and the barium and lithium ions were removed as the insoluble carbonates. The resulting solution was taken to dryness and combined with the organic extracts to give a syrup (241 mg.). The reduced aldobiouronic acid was remethylated with methyl iodide and silver oxide, and the methylated disaccharide (259 mg.) was hydrolysed with 0·8N-hydrochloric acid (20 ml.) at 100° for 6 hr. and after neutralisation gave a syrup (208 mg.). Chromatographic examination of the hydrolysate showed two main conponents, tetra-*O*-methylglucose and 3 : 4-di-*O*-methylxylose, together with traces of 2 : 3 : 4-tri-*O*-methyl- and 3-*O*-methyl-xylose, and 2 : 3 : 4-tri-*O*-methylglucose; no 2 : 3-di-*O*-methylxylose could be detected. The main components were separated on filter sheets with solvent B, to yield 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, m. p. [after recrystallisation from light petroleum (b. p. 40—60°) containing a little ether] and mixed m. p. 95·5—96·5°, and 3 : 4-di-*O*-methyl-D-xylose, identified by conversion into 3 : 4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 64—66°.

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744. *The Constitution of a Wheat-straw Hemicellulose.*

By G. O. Aspinall and Eric G. Meek.

A wheat-straw hemicellulose, containing uronic acid residues (*ca.* 3%), gave on hydrolysis xylose and arabinose in the ratio of 11 : 1. Hydrolysis of the methylated polysaccahride yielded 2 : 3 : 5-tri-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, and 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, together with some 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose. The structure of the polysaccharide is discussed in the light of these and other results.

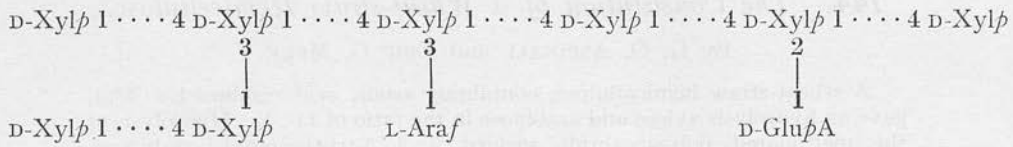
Previous structural investigations have indicated that a variety of closely related polysaccharides constitute wheat-straw hemicellulose. Adams [1] and Bishop [2] showed that the hemicellulose contains a backbone of 1 : 4-linked β-D-xylopyranose residues, to which are attached through position 3 a number of L-arabofuranose and D-glucopyruronic acid residues as single-unit side-chains. Earlier studies in these laboratories [3] showed that it was possible to fractionate the hemicellulose and to isolate a xylan, devoid of arabinose residues, but still containing uronic acid residues as an integral part of the molecular structure. These investigations were carried out on hemicellulose fractions isolated by alkaline extraction from straw which had previously been treated with acidified sodium chlorite solution to remove lignin. In view of the possibility of degradation of polysaccharides during chlorite delignification,[4] we have now examined the hemicellulose extracted directly with alkali from wheat straw rather than from the holocellulose.

The hemicellulose was extracted from straw, previously extracted with benzene and methanol, by the procedure used in the extraction of beechwood hemicellulose A,[5] the structure of which we have previously studied.[6] The polysaccharide contained uronic acid residues (*ca.* 3%) and yielded on hydrolysis xylose and arabinose in the ratio of 11 : 1. Graded hydrolysis liberated arabinose preferentially, suggesting that this sugar occurred in the furanose form, but selective removal of these labile residues to yield a xylan devoid of arabinose residues could not be achieved without extensive degradation of the molecule, as appreciable quantities of xylose and xylose-containing oligosaccharides were released before all the arabinose residues had been removed. On more vigorous hydrolysis, a resistant acidic fraction was isolated; reduction of the derived methyl ester methyl glycoside with potassium borohydride, followed by hydrolysis, yielded 4-*O*-methylglucose, glucose, and xylose, indicating the presence in the polysaccharide of residues of glucuronic acid (partly as the 4-methyl ether).

The xylan was converted into its fully methylated derivative, hydrolysis of which yielded the following methylated sugars, characterised by the formation of crystalline derivatives : 2 : 3 : 5-tri-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3-di-*O*-methyl-D-xylose, and 2-*O*-methyl-D-xylose (in the approximate ratio of 3 : 1 : 41 : 4). Small quantities of 3-*O*-methyl-D-xylose were also present; these probably arose from hydrolysis of some of the methylated aldobiouronic acid. In addition, 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid was characterised and an acidic fraction, consisting mainly of a tetra-*O*-methylaldobiouronic acid, was isolated. Insufficient of the latter fraction was isolated for a complete identification, but the following chromatographic studies leave little doubt concerning the structure of the main component. Reduction of the derived methyl ester methyl glycoside with lithium aluminium hydride, followed by hydrolysis, gave 2 : 3 : 4-tri-*O*-methylglucose and 3-*O*-methylxylose (clearly distinguished from the 2-methyl isomer by paper ionophoresis). Further methylation of the reduced acidic fraction, followed by hydrolysis, yielded 2 : 3 : 4 : 6-tetra-*O*-methylglucose and 3 : 4-di-*O*-methylxylose, together with smaller

amounts of 2 : 3 : 4-tri- and a mono-*O*-methylxylose. It follows that the tetra-*O*-methylaldobiouronic acid was 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methylglucuronosyl)xylose. It is probable that the acidic fraction contained traces of a methylated aldotriouronic acid in addition to the tetra-*O*-methylaldobiouronic acid.

Although no unique structure can be advanced for this xylan, the results are consistent with the general type of structure shown :



In agreement with other workers,[1, 7, 8] we are of the opinion that the L-arabinose residues are integral parts of the molecule, occurring exclusively as end-groups in the furanose form. It is probable that these residues occur as single-unit side-chains linked to the backbone of β-1 : 4-D-xylopyranose residues through position 3 of xylose; direct proof for this mode of attachment has been provided by Bishop,[9] who isolated the trisaccharide, *O*-L-arabofuranosyl-(1→3)-*O*-D-xylopyranosyl-(1→4)-D-xylopyranose, from the enzymic hydrolysis of wheat-straw hemicellulose. The methylated aldobiouronic acid isolated from the hydrolysis of the methylated xylan shows that the D-glucuronic acid residues (partially present as the 4-methyl ether) are linked directly to the main chain through position 2 of xylose; previously, 1 : 3-linkages were reported for the aldobiouronic acid units in wheat-straw xylans.[2,3] Although the evidence for a 1 : 3-linked aldobiouronic acid unit in the arabinose-free xylan from wheat straw [3] rested solely on the chromatographic distinction between 2- and 3-*O*-methylxylose, it will be recalled that Bishop [2] provided conclusive proof for the structure of the aldobiouronic acid which he isolated from the hydrolysis of wheat straw. It seems probable, therefore, that the several wheat-straw xylans differ amongst themselves as to the mode of attachment of glucuronic acid to xylose residues, and, indeed, in some fractions [7] glucuronic acid residues appear to be absent. The 1 : 2-linked aldobiouronic acid unit found in this xylan is a common structural feature of wood hemicelluloses [6, 10, 11] and has also been encountered in oat-straw xylan.[12]

A molecular-weight determination by the isothermal-distillation method (by the courtesy of Dr. C. T. Greenwood and Mr. W. N. Broatch) gave a value of 11,800 ± 400 (degree of polymerisation, 71—76) for the methylated xylan. This value, taken together with the value of one non-reducing end-group per *ca.* 50 residues, suggests that some of the xylan chains are branched, probably through position 3. In this respect, this xylan differs from the arabinose-free xylan,[3] also isolated from wheat straw but after chlorite delignification, in which no evidence for branching was obtained. However, Bishop [13] has obtained evidence for branching in some wheat-straw hemicullulose fractions by the isolation of non-linear xylose-containing oligosaccharides on partial acid hydrolysis.

It is clear from these and previous results that many closely related polysaccharides may be derived from wheat straw; it is not certain, however, that all these different molecular species exist side by side in the same plant. Although it is possible that some of these may have arisen from the use of different methods of extraction, our earlier studies [3] have shown that xylans, differing significantly in the proportion of arabinose residues present, may be obtained by the fractionation of a particular wheat-straw hemicellulose. The present situation with regard to the detailed structure of wheat-straw xylan re-emphasises the need for using the mildest possible methods for the extraction of polysaccharides from plant cell wall and for developing more selective methods for the fractionation of mixtures of polysaccharides.

Experimental

Unless otherwise stated paper chromatography was carried out on Whatman No. 1 filter paper with the upper layers of the following solvent systems (v/v) : (A) butan-1-ol–ethanol–water (4 : 1 : 5); (B) benzene–ethanol–water (167 : 47 : 15); (C) butan-1-ol–acetic acid–water

(4 : 1 : 5); (D) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (E) pentan-1-ol–ethanol–water (2 : 1 : 2). Extractions and reactions involving the use of alkali were performed, as far as possible, under nitrogen.

Isolation of Wheat-straw Xylan.—Wheat straw, previously extracted with benzene and methanol, was ground in a " Raymond " laboratory mill, and the finely ground straw (150 g.; moisture content, 9·4%) was extracted successively with aqueous sodium hydroxide of increasing concentration (0·01N, 2 l. + 2 × 1·5 l.; 0·1N, 1·5 l.; N, 3 × 1·5 l.). The extracts with N-sodium hydroxide were acidified with glacial acetic acid to pH 4, and the precipitates (A) thus obtained were separated at the centrifuge. Further precipitates (B) were obtained on addition of an equal volume of ethanol to the supernatant liquors. A qualitative chromatographic examination of the sugars given on hydrolysis of the precipitates A and B indicated that the same sugars were present, so the polysaccharides were combined. The polysaccharide (37·3 g.) was purified by three precipitations from alkaline solution by the addition of acetic acid and ethanol. The purified polysaccharide had $[\alpha]_D^{19}$ −91·7° (*c*, 0·5 in N-sodium hydroxide) [Found : lignin, 7·2%; ash, 0·87%; uronic anhydride, 2·95% (by decarboxylation); OMe, 1·5%]. Chromatographic examination of the hydrolysate by Hirst and Jones's [11] method indicated the presence of xylose (90·6%) and arabinose (8·4%).

Acid Hydrolysis of Xylan.—(a) *Graded hydrolysis.* A suspension of xylan in 0·02N-oxalic acid was heated at 100°; samples were withdrawn at intervals, an equal volume of ethanol was added, and the precipitate was removed at the centrifuge. The precipitate was hydrolysed with 2N-sulphuric acid, and the hydrolysate was examined on the chromatogram with solvent A. The supernatant liquid was neutralised with barium carbonate and examined directly on the chromatogram. The results indicated that arabinose was rapidly released but that before all the arabinose residues were removed from the remaining polysaccharide (2 hr.) appreciable quantities of xylose and xylose-containing oligosaccharides could be detected in the supernatant liquid.

(b) *Examination of the acidic components in the hydrolysate.* Xylan (2·3 g.) was treated with 2N-sulphuric acid (50 ml.) at room temperature for 22 hr. and then heated at 100° for 4·5 hr.; the insoluble residue which remained was treated with 2N-sulphuric acid (50 ml.) at 100° for a further 5 hr. The combined hydrolysates were neutralised with barium carbonate, and the filtrate was de-ionised by treatment with Amberlite resin IR-120(H) and concentrated to a syrup (2·0 g.), which contained xylose, arabinose, glucose (trace), and acidic oligosaccharides. The syrup was fractionated on acid-washed charcoal–celite (1 : 1, w/w) (42 × 1·8 cm.), elution with water yielding monosaccharides together with a small quantity of acidic material (fraction 1), and elution with ethyl methyl ketone–water (5 : 95, v/v) yielding acidic oligosaccharides together with a trace of xylose (fraction 2). The acidic components from fraction 1 were separated on filter sheets with solvent D and combined with fraction 2. The acids were converted into the methyl ester methyl glycosides, reduced with potassium borohydride, and hydrolysed, chromatography showing 4-*O*-methylglucose, glucose, and xylose.

Methylation of Xylan.—Xylan (20·5 g.) was methylated fifteen times with methyl sulphate and sodium hydroxide. The fraction (5·1 g.) soluble in boiling chloroform–light petroleum (b. p. 60—70°) (9 : 11, v/v) but insoluble in boiling chloroform–light petroleum (1 : 4, v/v) was further methylated with methyl iodide and silver oxide to give a brown glass (4·77 g.) (Found : OMe, 36·0%). This material was fractionally precipitated from chloroform solution by the addition of light petroleum (b. p. 60—70°), and the fraction precipitated in light petroleum–chloroform (4 : 1) but not by light petroleum–chloroform (3 : 1 v/v) was used in subsequent experiments (Found : OMe, 38·3%).

Hydrolysis of Methylated Xylan and Separation of Methylated Sugars.—Methylated xylan (3·54 g.) was hydrolysed successively with boiling methanolic 1% hydrogen chloride (400 ml.) for 6 hr. ($[\alpha]_D$ +58·7°, constant) and 0·5N-hydrochloric acid (200 ml.) for 7·5 hr. ($[\alpha]_D$ +18·6°, constant). After neutralisation with silver carbonate, the hydrolysate was taken to dryness to yield a syrup (3·75 g.). Quantitative paper chromatography [15] in solvent A showed the presence of tri-*O*-methylpentose (tri-*O*-methylarabinose and tri-*O*-methylxylose) (10·8%), di-*O*-methylxylose (75·5%), and mono-*O*-methylxylose (10·8%), together with small quantities of acidic substances. Quantitative chromatography in solvent B showed that tri-*O*-methylarabinose and tri-*O*-methylxylose were present in the ratio of 3 : 1. The syrup (3·5 g.) was fractionated on cellulose [16] (93 × 2·7 cm.) by elution with solvent B to yield seven fractions, and two further fractions were obtained by elution with butan-1-ol 50% saturated with water, and with water.

Fraction 1. The syrup (1 mg.) travelled on the chromatogram in solvent B at the same rate as 2 : 3 : 5-tri-*O*-methyl-L-arabinose and was combined with fraction 2*a*.

Fraction 2. Chromatographic examination of the syrup (282 mg.) in solvent B showed the presence of two components, which were separated on filter sheets (Whatman 3MM) with solvent B, to give fractions 2*a* and 2*b*. Fraction 2*a* had n_D^{15} 1·4516 and was identified as 2 : 3 : 5-tri-*O*-methyl-L-arabinose by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. 134°. Fraction 2*b* crystallised and had m. p. and mixed m. p. (with 2 : 3 : 4-tri-*O*-methyl-D-xylose) 91—92° and $[\alpha]_D^{16}$ +18·2° (equil.) (*c*, 0·55 in H_2O). The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine did not crystallise, but chromatographic examination of the syrup in butan-1-ol–pyridine–water (10 : 3 : 3) showed two components (corresponding to the glycosylamine and the parent sugar [17]) having the same rates of movement as those derived from an authentic sample.

Fraction 3. The syrup (4 mg.) travelled on the chromatogram in solvent B at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-xylose and was combined with fraction 2*b*.

Fraction 4. The syrup (2·8 g.), when seeded with 2 : 3-di-*O*-methyl-α-D-xylose, crystallised as the β-anomer (full details have been published previously [18]). The identity of the sugar was confirmed by conversion into the aniline derivative, m. p. and mixed m. p. 123°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 134°.

Fractions 5 *and* 7. Chromatographic examination of the syrup (105 mg.) indicated the presence of a major component having R_{rhamnose} 1·64—1·78 in solvent C and giving a dipolar spot similar to that given by the acidic component isolated from the hydrolysis of methylated pear cell wall xylan,[19] together with small quantities of di-*O*-methylxylose. Reduction of the derived methyl glycoside methyl ester with lithium aluminium hydride, followed by hydrolysis, yielded sugars identified chromatographically as mono- and di-*O*-methylxylose and 2 : 3 : 4-tri-*O*-methylglucose.

Fraction 6. Chromatographic examination of the syrup (22 mg.) gave the same dipolar spot, with characteristic orange fluorescence in ultraviolet light, as that given by fractions 5 and 7. On hydrolysis with 2N-sulphuric acid at 100° for 44 hr., the slower-moving component decreased in amount, whilst the faster-moving component, which travelled at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, increased, and mono-*O*-methylxylose was liberated. Complete hydrolysis was achieved by 2N-sulphuric acid at 100° in a sealed tube in 66 hr.; the products were separated on filter sheets with solvent C. The first component was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid by conversion into the β-methyl glycoside, m. p. 130—132°. Ionophoretic examination of the mono-*O*-methylxylose showed it to be mainly the 3-methyl ether with small quantities of the 2-methyl ether.

Fraction 8. The fraction (227 mg.) partially crystallised. The crystalline portion was ionophoretically homogeneous and had m. p. and mixed m. p. (with 2-*O*-methyl-D-xylose) 131—132° and $[\alpha]_D^{15}$ +28·9° (equil.) (*c*, 2·4 in H_2O). The identity of the sugar was confirmed by conversion into 2-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 128—129°. The syrupy portion was shown ionophoretically to consist mainly of the 2-methyl ether with small amounts of the 3-methyl ether.

Fraction 9. The syrup (118 mg.) was chromatographically similar to fractions 5—7, but also contained traces of slow-moving components. Chromatography on filter sheets in solvent C yielded a purified acidic fraction, having $[\alpha]_D^{18}$ +10·2° (*c*, 0·1 in H_2O). The derived methyl ester methyl glycoside was reduced with lithium aluminium hydride, and the product was divided into two portions. The first portion was hydrolysed with 0·5N-hydrochloric acid at 100° for 8 hr., and quantitative chromatography [15] showed 2 : 3 : 4-tri-*O*-methylglucose and mono-*O*-methylxylose to be present in the ratio of 1 : 1. The second portion was methylated twice with silver oxide and methyl iodide, and chromatography of the hydrolysate in solvents B and E showed 2 : 3 : 4 : 6-tetra-*O*-methylglucose, 2 : 3 : 4-tri-*O*-methylxylose, 3 : 4-di-*O*-methylxylose, and mono-*O*-methylxylose.

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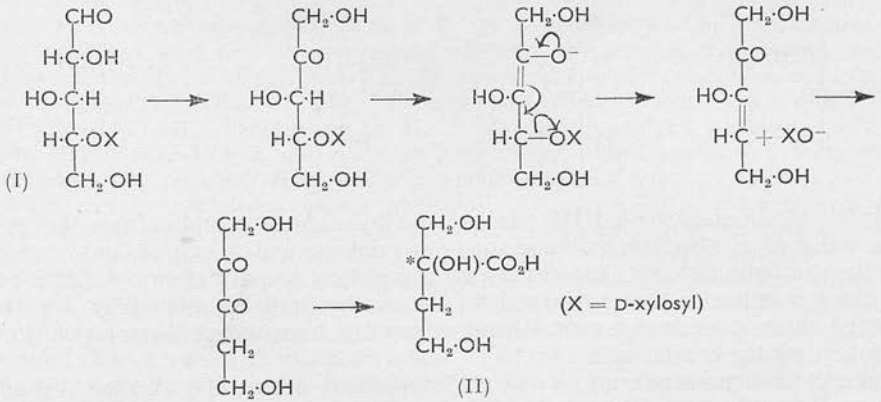
938. *The Degradation of Xylobiose and Xylotriose by Alkali.*

By G. O. Aspinall, (Miss) Mary E. Carter, and (in part) M. Los.

The 1 : 4-lactone of (±)-2 : 4-dihydroxy-2-hydroxymethylbutanoic acid (xylo*iso*saccharinolactone) has been synthesised from 1 : 4-diacetoxybutan-2-one. The alkaline degradation of xylobiose and xylotriose has been studied, and xylo*iso*saccharinolactone has been identified as a major degradation product.

The xylans, which constitute the major fraction (other than cellulose) of the cell-wall polysaccharides of many land plants, normally require the use of alkaline solutions for their removal from the plant. Structural studies [1] have shown that all the xylans so far examined from land plants contain a backbone of 1 : 4-linked β-D-xylopyranose residues, but that the several xylans differ in the number, nature, and mode of linkage of the sugar residues attached as side-chains. We have little knowledge, however, of the extent to which these polysaccharides may have undergone structural modification during their isolation by a process analogous to the alkaline degradation of reducing sugars.[2] We now report on the alkaline degradation of 4-*O*-β-D-xylopyranosyl-D-xylopyranose (xylobiose) (I) and xylotriose, oligosaccharides chosen as model compounds containing the 1 : 4-linkages typical of the xylans. Since the appearance of a preliminary account [3] of some of the following results, Whistler and Corbett [4] have published the results of a similar investigation.

It would be expected from work by Corbett, Kenner, and Richards [2] that 1 : 4-linked xylo-oligosaccharides [*e.g.* xylobiose (I)] would be degraded by alkali with the formation of xylo*iso*saccharinic acid (II). This acid contains only one asymmetric carbon atom, marked *, and if degradation proceeds by the type of mechanism indicated involving a benzilic acid rearrangement in the final stage a racemic product would be expected. To facilitate subsequent studies this acid was synthesised. 1 : 4-Diacetoxybutan-2-one, prepared by the hydration of butyne-1 : 4-diol diacetate,[5] was converted into the cyano-



(I) (II) (X = D-xylosyl)

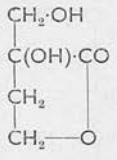
hydrin; hydrolysis of the crude cyanohydrin, which was accompanied by removal of the acetyl groups and lactonisation, yielded the 1 : 4-lactone (III) of (±)-2 : 4-dihydroxy-2-hydroxymethylbutanoic acid (II). The structure of the lactone was confirmed by the isolation of formaldehyde and α-tetronic acid [6] on periodate oxidation.

Although most recent studies of the alkaline degradation of carbohydrates [2] have involved the use of lime-water, we have investigated the action of *ca.* N-sodium hydroxide (in the absence of oxygen) on xylobiose (I) and xylotriose in order to simulate the conditions normally employed in the extraction of xylans from lignified tissues. Preliminary experiments showed that the reaction patterns were similar with both reagents and we have

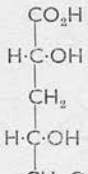
already reported the isolation of the lactone (III) from the reaction of lime-water on xylobiose.[3] Paper chromatography showed that xylobiose when treated with alkali produced four lactones, one of which travelled at the same rate as the synthetic lactone (III). The other three lactones were also detected as products of the alkaline degradation of D-xylose and it is probable that these were the lactones of the isomeric "*erythro*"- and "*threo*"-D-2 : 4 : 5-trihydroxypentanoic acids (IV and V) and of 2 : 4-dihydroxybutanoic acid (VI) isolated by Nef from the action of 8N-sodium hydroxide on D-xylose.[7] The *iso*lactone (III) could not be detected amongst the degradation products of D-xylose. When an acid-degraded esparto xylan was treated with N-sodium hydroxide, chromatographic examination of the products showed the presence of the *iso*lactone and an unknown substance which moved more slowly on the chromatogram; the three lactones known to be formed from xylose could not be detected. The unknown substance was an ester or lactone which yielded xylose and the *iso*lactone on hydrolysis; insufficient of this compound was available for a full characterisation, so that its importance as a product cannot be assessed. It is possible, however, that the compound was a reversion product derived from xylose and the *iso*lactone as a chromatographically similar substance was shown to be produced when an alkaline solution of the acid (II) and D-xylose was de-ionised with cation-exchange resin and concentrated; as no lactones of the acids (IV), (V), and (VI) were detected during the xylan degradation it is unlikely that xylose was formed as a reaction intermediate, but it is possible that xylose might have been produced by the hydrolysis of unchanged xylan during the concentration of the acidic products. The absence of the lactonisable acids (IV), (V), and (VI) during the xylan degradation suggests that these straight-chain acids arise only from xylose, and that their presence amongst the degradation products of xylobiose and xylotriose indicates the formation of xylose in the course of the reaction of these oligosaccharides with alkali—xylose, indeed, was detected chromatographically in these reactions. This suggests that the degradation of xylobiose and xylotriose with alkali involves a "peeling" reaction [2] of the type

$$\text{xylotriose} \longrightarrow \text{xylobiose} \longrightarrow \text{xylose}$$

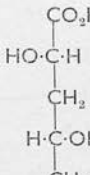
in which each successive reducing sugar residue gives rise to acidic products with the simultaneous exposure of a new reducing group.



(III)



(IV)



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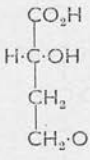

(VI)

Xylo*iso*saccharinolactone (III) was isolated by chromatography from the products of the action of *ca.* N-sodium hydroxide on both xylobiose and xylotriose, and was identical with the synthetic lactone. In neither case was optical activity observed. It is possible that the low optical activity observed for the similar product isolated by Whistler and Corbett [4] either arose from a contaminant or resulted from a partial resolution during its separation *via* the brucine salt.

Quantitative measurements of the acids produced during the alkaline degradations were carried out by a modification [8] of Bamford, Bamford, and Collins's method,[9] in which the lactonisable and non-lactonisable acids were differentiated. From the quantities of lactonisable acids formed from xylose, xylobiose, and xylotriose (0·35, 0·75, and 1·16 mol., respectively), taken together with the quantities of xylo*iso*saccharinolactone isolated from xylobiose and xylotriose (0·38 and 0·82 mol.), it is reasonable to conclude that the same quantities of acids (IV), (V), and (VI) are produced in each case, and that the additional quantities of lactonisable acid formed from xylobiose and xylotriose can be accounted for in terms of the *iso*saccharinolactone. Such an observation is again consistent with a "peeling" mechanism for the alkaline degradation of xylobiose and xylotriose. It is clear, however, from these quantitative measurements that the degradations also result

in the formation of non-lactonisable acids and that formation of saccharinic acid is not the only reaction in which glycosidic bonds are broken with the exposure of new reducing groups.

Experimental

Paper-partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v) : (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) butan-1-ol–formic acid–water (500 : 115 : 385; top layer); and (C) ethyl acetate–acetic acid–water (10 : 1·3 : 1).

1 : 4-*Lactone of* (±)-2 : 4-*Dihydroxy*-2-*hydroxymethylbutanoic Acid* (*Xyloisosaccharinolactone*).—1 : 4-Diacetoxybutan-2-one [5] (15·9 g.) was shaken with a solution of sodium metabisulphite (16 g.) in water (40 ml.), and a solution of potassium cyanide (10 g.) in water (25 ml.) then added slowly with shaking during 0·5 hr., the temperature being maintained at 0°. The mixture was extracted with ethyl acetate and the extract was evaporated. The resulting syrup was hydrolysed with a mixture of concentrated hydrochloric acid (20 ml.) and water (30 ml.) at 60° for 2 hr. and set aside overnight at room temperature. Acid was removed by distillation, the dry residue was extracted with ethyl acetate, and the extract filtered; after partial removal of solvent the 1 : 4-*lactone* of 2 : 4-*dihydroxy*-2-*hydroxymethylbutanoic acid* (6·2 g.) crystallised on cooling. After recrystallisation from ethyl acetate the lactone (5·8 g.) had m. p. 95·5—96·5° (Found : C, 45·6; H, 6·2. $C_5H_8O_4$ requires C, 45·5; H, 6·1%).

Periodate Oxidation of Xyloisosaccharinolactone.—(a) *Identification of formaldehyde.* Sodium metaperiodate solution (5 ml.; *ca.* 0·3M) was added to a solution of the lactone, and set aside overnight. Excess of periodate was destroyed according to Bell's procedure [10] and addition of dimedone (160 mg.) in ethanol (2 ml.) yielded the formaldehyde derivative, m. p. and mixed m. p. 189—190°.

(b) *Identification of α-tetronic acid.* Sodium metaperiodate solution (1·78 g. in 175 ml. of water) was added slowly during 6 hr. to a solution of the lactone (1·11 g.) in water (25 ml.). Next morning the solution was extracted with ether for 6 hr. Evaporation yielded a solid which, after four sublimations and two recrystallisations from benzene, had m. p. 109—110° and mixed m. p. 106—109° (with α-tetronic acid, synthesised by Schinz and Hinder's method; [6] m. p. 105—106°).

Preparation of Xylobiose and Xylotriose.—Esparto hemicellulose (50 g.) was shaken in water (750 ml.) for 24 hr., water (3·25 l.) and N-sulphuric acid (1 l.) were added, and the mixture was heated on a boiling-water bath for 100 min. The cooled solution was neutralised with sodium hydrogen carbonate and set aside for 48 hr. at 4°. A small quantity of degraded esparto xylan separated and was used in a subsequent experiment. The solution was concentrated and poured on charcoal–Celite (1 : 1, 56 × 7 cm.); elution with water removed monosaccharides (xylose together with small amounts of arabinose) and inorganic salts. Elution with ethanol–water (1 : 19; 13 l.) and ethanol–water (3 : 17; 6 l.) yielded fractions 1 (4·2 g.) and 2 (2·5 g.), consisting largely of xylobiose and xylotriose respectively. Final purifications were effected by chromatography on filter sheets (Whatman 4MM), solvent A being used. A sample of xylobiose was crystallised from aqueous methanol containing a little light petroleum (b. p. 60—80°) and had m. p. and mixed m. p. 183—187°. The xylotriose was characterised by conversion into the octa-acetate, m. p. 110° (Whistler and Tu [11] report m. p. 109—110°).

Determination of Acids Produced by the Action of Alkali on Xylose, Xylobiose, and Xylotriose.—Chromatographically pure sugars were dissolved in carbonate-free sodium hydroxide solution (25 ml.) under oxygen-free nitrogen. The mixture was set aside at room temperature (16—18°), and samples (2 ml.) were withdrawn periodically and were de-ionised by passing through a column (35 × 1·2 cm.) of Amberlite resin IR-120(H) (*ca.* 25 g.). The column was then washed with water (30 ml.), and the eluate was titrated potentiometrically with carbonate-free sodium

Sugar degraded	Time (days)	Acid (equiv./mole) total	lacton-isable	non-lacton-isable
Xylose (0·079M in 1·1N-NaOH)	3	0·74	0·26	0·48
	5	1·10	0·30	0·80
	7	1·14	0·35	0·79
Xylobiose (0·032M in 0·88N-NaOH)	1	0·55	0·31	0·24
	3	1·30	0·65	0·65
	5	1·80	0·75	1·05
	7	2·15	0·67	1·47
Xylotriose (0·0569M in 0·88N-NaOH)	1	0·78	0·59	0·19
	3	1·50	0·69	0·81
	5	2·12	0·96	1·16
	7	2·54	1·08	1·45
	9	2·84	1·10	1·74
	16	3·03	1·16	1·87

hydroxide (*ca.* 0·1N). After the electrodes had been washed, the total solution was acidified with hydrochloric acid (0·5 ml.; *ca.* N) and concentrated to *ca.* 15 ml. The solution was rapidly cooled and kept at room temperature for 15 min., and the non-lactonisable acids were titrated potentiometrically with carbonate-free sodium hydroxide. All operations were carried out in a nitrogen atmosphere.

Paper Chromatography of Saccharinic Acids.—Alkaline solutions were de-ionised by treatment with Amberlite resin IR-120(H) and the resulting solutions were evaporated. Saccharinic acids were detected on the chromatogram as lactones by spraying the paper with hydroxylamine followed by acidic ferric chloride as described by Abdel-Akher and Smith.[12] The lactones derived from D-xylose had R_X values of 0·62, 0·81, and 1·09 in solvent C and 0·74, 0·86, and 1·07 in solvent B (x = xylo*iso*saccharinolactone). These three lactones in addition to the *iso*-lactone were detected as degradation products from xylobiose and xylotriose.

When acid-degraded esparto xylan was treated with 1·03N-sodium hydroxide in an atmosphere of nitrogen for 28 days, paper chromatography showed the presence of two substances, giving a colour with hydroxylamine and ferric chloride and having R_X values of 0·51 and 1·0 in solvent B. A small sample of the slower-moving component was separated on filter sheets, solvent B being used; hydrolysis with 0·5N-sulphuric acid yielded the *iso*lactone and xylose, both detected chromatographically. An alkaline solution of D-xylose and the synthetic lactone was de-ionised with Amberlite resin IR-120(H) and concentrated to a syrup; chromatography showed substances having R_X values of 0·51 and 1·0.

*Isolation of Xylo*iso*saccharinolactone from the Alkaline Degradation of Xylobiose and Xylotriose.*—A solution of xylobiose (0·88 g.) in 1·1N-sodium hydroxide (carbonate-free) (35 ml.) was set aside at room temperature for 10 days in an atmosphere of nitrogen. The solution was de-ionised by passing it through Amberlite resin IR-120(H) (75 g.) and concentrated to a syrup which was fractionated on filter sheets with solvent B. Extraction of the appropriate sections of the papers yielded a chromatographically pure syrup (0·171 g.) which was crystallised from ethyl acetate to give xylo*iso*saccharinolactone, m. p. and mixed m. p. 94°, $[\alpha]_D$ 0° (*c*, 2·8 in H_2O). In a similar experiment xylotriose (0·414 g.) yielded xylo*iso*saccharinolactone (0·134 g.), m. p. and mixed m. p. 94—96°, $[\alpha]_D$ 0° (*c*, 1·8 in H_2O).

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436. *Selective Esterification of Equatorial Hydroxyl Groups in the Synthesis of Some Methyl Ethers of* D-*Mannose.*

By G. O. Aspinall and G. Zweifel.

Methyl 4 : 6-*O*-ethylidene-α-D-mannoside (A) and 1 : 6-anhydro-β-D-mannopyranose (B) (and its 4-methyl ether), compounds for which only one chair conformation is possible, undergo selective esterification at their equatorial hydroxyl groups. By employing these reactions, 3-*O*- and 3 : 4-di-*O*-methyl-D-mannose have been synthesised and a new synthesis of 2-*O*-methyl-D-mannose has been achieved.

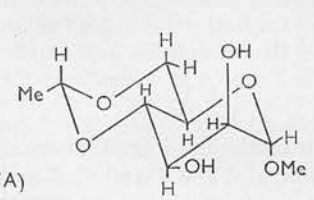
By conventional methods, syntheses of partially methylated derivatives of D-mannose [1] have been carried out in which the hydroxyl groups in the 4 and 6, 2 and 3, and the 6 positions have been blocked. In only two cases, namely, in the syntheses of 2-*O*- [2] and 3 : 4 : 6-tri-*O*- [3] methyl-D-mannose, has it been possible to differentiate between the hydroxyl groups at $C_{(2)}$ and $C_{(3)}$. It is, however, well established that in *cyclo*hexane systems [4] equatorial secondary hydroxyl groups are more readily esterified by derivatives of carboxylic acids than are axial hydroxyl groups. We have now shown that similar preferential reactivity obtains with some sugar derivatives, of whose most stable conformations there is little doubt. Starting with methyl 4 : 6-*O*-ethylidene-α-D-mannoside (A) and 1 : 6-anhydro-β-D-mannopyranose (B) (and its 4-methyl ether), in which the D-mannopyranose rings are held in the C 1 and the 1 C conformation,[5] respectively, and by employing these selective reactions, 2- and 3-mono-*O*-, and 3 : 4-di-*O*-methyl-D-mannose have been synthesised.

Methyl 4 : 6-*O*-ethylidene-α-D-mannoside (A) [6] reacts with 1 mol. of toluene-*p*-sulphonyl chloride to give the 3-toluene-*p*-sulphonate (I) in good yield; the 2-toluene-*p*-sulphonate (VIII) could not be detected. Methylation of the 3-toluene-*p*-sulphonate (I) followed by removal of the ester group with sodium amalgam gave methyl 4 : 6-*O*-ethylidene-2-*O*-methyl-α-D-mannoside (III), hydrolysed to 2-*O*-methyl-α-D-mannose (IV). This sugar and its phenylhydrazone had similar physical constants to those reported by Pacsu and Trister.[2] The absence of a methoxyl group at $C_{(3)}$ was indicated as the derived syrupy mixture of methyl pyranosides consumed 1 mol. of periodate; the presence of methoxyl groups in positions other than 2 and 3 was precluded by the mode of synthesis.

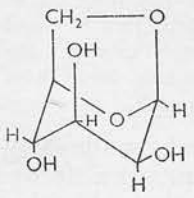
It is of interest to compare the selective tosylation of methyl 4 : 6-*O*-ethylidene-α-D-mannoside (A) with that of methyl 4 : 6-*O*-benzylidene-α-D-glucoside.[7] The latter compound, in which the hydroxyl groups at $C_{(2)}$ and $C_{(3)}$ are both equatorial, reacts preferentially with toluene-*p*-sulphonyl chloride at position 2. Whatever explanation, steric or electronic, may be advanced for this selective reactivity, it is clear that the preferential reactivity of the equatorial 3-hydroxyl group in the D-mannose derivative can be explained on stereochemical grounds.

In the synthesis of 3-*O*-methyl-D-mannose (X), nitrate ester formation was used for the selective blocking of the 3-hydroxyl group. Selective esterification was not so marked in this case as it was necessary to use a large excess of nitrating reagent, but it was possible to modify the conditions used by Honeyman and Morgan [6] in the preparation of methyl 4 : 6-*O*-ethylidene-α-D-mannoside 2 : 3-dinitrate so that a reasonable quantity of the 3-nitrate (V) could be isolated; no 2-nitrate was detected. The structure of the 3-nitrate was proved by methylation followed by reductive removal of the nitrate ester to give methyl 4 : 6-*O*-ethylidene-2-*O*-methyl-α-D-mannoside (III). Tosylation of the 3-nitrate

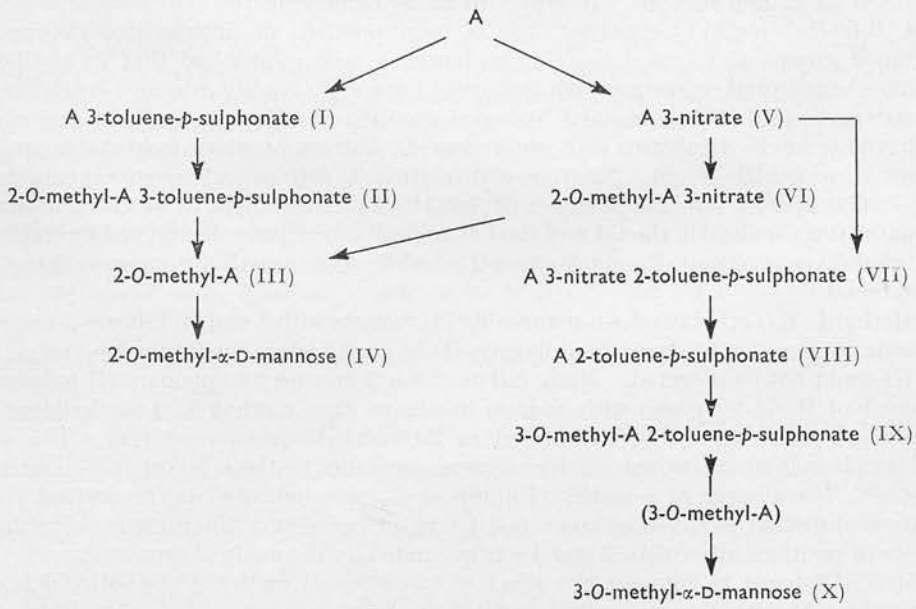
(V) followed by reductive denitration and methylation gave methyl 4 : 6-*O*-ethylidene-3-*O*-methyl-α-D-mannoside 2-toluene-*p*-sulphonate (IX). The axial toluene-*p*-sulphonate grouping in this compound was very resistant to reductive cleavage with lithium aluminium hydride (reaction was incomplete after 20 hr. in refluxing tetrahydrofuran), but the grouping was removed smoothly with sodium amalgam. Bollinger and Ulrich [8] have reported similar difficulties in the removal of the toluene-*p*-sulphonate group from methyl 4 : 6-*O*-benzylidene-3-*O*-methyl-α-D-altroside 2-toluene-*p*-sulphonate with lithium aluminium hydride; here also the ester grouping is axial. Hydrolysis of the syrupy product afforded crystalline 3-*O*-methyl-α-D-mannose (X). The structure of the sugar was confirmed by its conversion into 3-*O*-methyl-D-glucosazone and by the fact that the derived syrupy mixture of methyl pyranosides did not react with periodate.



(A)



(B)

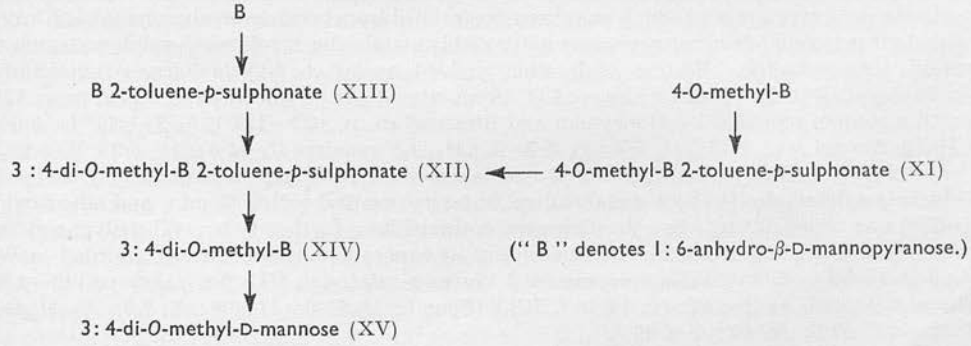


("A" denotes methyl 4 : 6-*O*-ethylidene-α-D-mannoside)

Honeyman and Stening [9] have also prepared the 3-toluene-*p*-sulphonate (I) and the 3-nitrate (V) (but by different methods), and the 3-nitrate 2-methyl ether (VI) and the 3-nitrate 2-toluene-*p*-sulphonate (VII) of methyl 4 : 6-*O*-ethylidene-α-D-mannoside (A). We are grateful to Dr. J. Honeyman for communicating to us the results of his investigations before publication and for supplying samples of these compounds for comparison.

The synthesis of 3 : 4-di-*O*-methyl-D-mannose depended on the preferential reactivity of the 2-hydroxyl group in 1 : 6-anhydro-β-D-mannopyranose (B) and in its 4-methyl ether. Reeves [10] has shown by complex formation with cuprammonium that despite considerable non-bonded interactions (the axial 3-hydroxyl group lies in 1 : 3-relationship with the 1 : 6-anhydro-ring) both these compounds exist in the chair (1 C) rather than in the boat (3 B) conformation. Our observations agree with this result as both the anhydrosugar (B) and its 4-methyl ether react with 1 mol. of toluene-*p*-sulphonyl chloride to give

the equatorial 2-toluene-*p*-sulphonate (XIII and XI, respectively) in good yield. Both compounds (XIII and XI) yielded 1 : 6-anhydro-3 : 4-di-*O*-methyl-β-D-mannopyranose (XII) on complete methylation. It is noteworthy that under certain conditions the 4-methyl ether (XI) may be isolated during methylation of 1 : 6-anhydro-β-D-mannopyranose 2-toluene-*p*-sulphonate (XIII). An examination of models shows clearly that the 3-hydroxyl group is considerably more hindered than the axial 4-hydroxyl group which lies below the plane of the ring, and is in 1 : 3-relationship only with the axial hydrogen atom. It would be expected, therefore, that the 4- would be more reactive than the 3-hydroxyl group. Treatment of 1 : 6-anhydro-3 : 4-di-*O*-methyl-β-D-mannopyranose 2-toluene-*p*-sulphonate with sodium amalgam readily gave 1 : 6-anhydro-3 : 4-di-*O*-methyl-β-D-mannopyranose (XIV), hydrolysis of which afforded 3 : 4-di-*O*-methyl-D-mannose (XV). The sugar, isolated as the monohydrate, was identical with a sample previously isolated [11] and on oxidation yielded the known 3 : 4-di-*O*-methyl-D-mannonolactone.



These experiments show clearly that marked differences exist between the reactivities of equatorial and axial hydroxyl groups in methyl 4 : 6-*O*-ethylidene-α-D-mannoside (A) and 1 : 6-anhydro-4-*O*-methyl-β-D-mannopyranose so that the equatorial hydroxyl groups may be selectively esterfied. A similar result was found with 1 : 6-anhydro-β-D-mannopyranose (B), although in this case analogy with certain disubstituted *cyclo*hexane derivatives [12] would suggest that the equatorial 2-hydroxyl flanked by axial substituents should be subject to greater steric compression and be less reactive than the 4-hydroxyl group. Without further knowledge of the chemistry of related compounds it is not yet possible to ascribe this result to electronic or steric factors. As far as we are aware the only example previously reported of this type of selective esterification in the carbohydrate group is the tosylation of the equatorial 3-hydroxyl group in 1 : 6-anhydro-2-*O*-benzoyl-β-D-altropyranose.[13] In this case, as with our examples, only one chair conformation is possible. It is probable that starting from stereochemically suitable compounds many partially substituted sugars, not readily accessible by other methods, may be synthesised by employing such preferential reactivity for the selective blocking of specific hydroxyl groups.

Experimental

Unless otherwise stated, chloroform solutions were dried (Na_2SO_4), evaporations were carried out under reduced pressure, and the light petroleum used had b. p. 60—80°. Chromatographic separations were carried out with columns of (*a*) activated alumina, Type H, 100/200 S mesh, supplied by Peter Spence and Sons, Ltd., and (*b*) as in (*a*) shaken with N-acetic acid, washed with water by decantation until free from acid, filtered, and dried at 260—280°. Paper partition chromatography was effected on Whatman No. 1 filter paper with (*a*) butan-1-ol–ethanol–water (4 : 1 : 5 v/v, upper layer) and (*b*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4) as solvents.

Methyl 4 : 6-O-*Ethylidene*-α-D-*mannoside*.—Methyl α-D-mannoside (10 g.), paraldehyde

(50 ml.), and concentrated sulphuric acid (0·08 ml.) were shaken at room temperature for 10 min. The solution was decanted into light petroleum (200 ml.)–saturated aqueous sodium carbonate (150 ml.). The residual methyl α-D-mannoside was treated four times with paraldehyde (25 ml.)–concentrated sulphuric acid (0·04 ml.) for periods of 10 min., and in each case the solution was decanted into the original light petroleum–sodium carbonate solution. Separation of the aqueous layer, after washing it with light petroleum (3 × 100 ml.), and evaporation yielded a white solid. Extraction of the solid with acetone and evaporation gave a syrup which crystallised from light petroleum–carbon tetrachloride (10 : 1). After three recrystallisations the mannoside (3·5 g.) had m. p. 112°, $[\alpha]_D^{17}$ +71° (*c*, 1·3 in $CHCl_3$). Honeyman and Morgan [6] give m. p. 117°, [α] +77° ($CHCl_3$).

Methyl 4 : 6-O-*Ethylidene*-α-D-*mannoside* 3-*Toluene*-p-*sulphonate* (I).—Toluene-*p*-sulphonyl chloride (2·6 g.) in pyridine (20 ml.) was added to methyl 4 : 6-*O*-ethylidene-α-D-mannoside (3 g.) in pyridine (20 ml.), and the solution was kept for 2 days at −5° and for 1 day at 0°. The solution was poured into aqueous sodium hydrogen carbonate; evaporation removed most of the pyridine. The mixture was extracted with chloroform and the chloroform extract was washed with dilute sulphuric acid, sodium hydrogen carbonate solution, and water. Concentration gave a syrup (4·5 g.) which was chromatographed from benzene on alumina (*a*). Elution with light petroleum–benzene gave a syrup (probably mainly the di-toluene-*p*-sulphonate) which would not crystallise. Elution with ether yielded *methyl* 4 : 6-O-*ethylidene*-α-D-*mannoside* 3-*toluene*-p-*sulphonate* (I) (3·1 g.), m. p. 129° (from ether–light petroleum) and mixed m. p. 125° (with a sample prepared by Honeyman and Stening,[9] m. p. 122—123°), $[\alpha]_D^{20}$ +25° (*c*, 0·6 in $CHCl_3$) (Found : C, 51·3; H, 5·9; S, 8·7. $C_{16}H_{22}O_8S$ requires C, 51·3; H, 5·9; S, 8·5%).

Methyl 4 : 6-O-*Ethylidene*-2-O-*methyl*-α-D-*mannoside* 3-*Toluene*-p-*sulphonate* (II).—The 3-toluene-*p*-sulphonate (I) (1 g.) was dissolved in boiling methyl iodide (5 ml.), and silver oxide (1·5 g.) was added during 5 hr.; heating was continued for a further 19 hr. The silver residues were filtered off and washed with chloroform. Evaporation of the filtrate afforded *methyl* 4 : 6-O-*ethylidene*-2-O-*methyl*-α-D-*mannoside* 3-*toluene*-p-*sulphonate* (II) (0·8 g.), m. p. 149—150° (from methanol), $[\alpha]_D^{18}$ +22° (*c*, 0·6 in $CHCl_3$) (Found : C, 51·9; H, 5·8; S, 7·9. $C_{17}H_{24}O_8S$ requires C, 52·5; H, 6·2; S, 8·0%).

Methyl 4 : 6-O-*Ethylidene*-2-O-*methyl*-α-D-*mannoside* (III).—Methyl 4 : 6-*O*-ethylidene-2-*O*-methyl-α-D-mannoside 3-toluene-*p*-sulphonate (0·7 g.) was dissolved in methanol–water (9 : 1; 25 ml.), sodium amalgam (4%; 10 g.) was added, and the mixture was stirred at 45° for 4 hr. and at room temp. for 20 hr. After being decanted from mercury the solution was neutralised with carbon dioxide, inorganic salts were filtered off, and the filtrate was evaporated. The residue was extracted with chloroform; evaporation gave a syrup which crystallised from ether–light petroleum to yield *methyl* 4 : 6-O-*ethylidene*-2-O-*methyl*-α-D-*mannoside* (III) (0·3 g.), m. p. 76—77°, $[\alpha]_D^{19}$ +42° (*c*, 0·6 in $CHCl_3$) (Found : C, 52·0; H, 7·6; OMe, 25·8. $C_{10}H_{18}O_6$ requires C, 51·3; H, 7·7%; OMe, 26·3%).

2-O-*Methyl*-α-D-*mannose* (IV).—Methyl 4 : 6-*O*-ethylidene-2-*O*-methyl-α-D-mannoside (1·6 g.) was heated with 0·5N-hydrochloric acid (25 ml.) at 100° for 6 hr. (constant rotation). The solution was neutralised with silver carbonate and filtered, hydrogen sulphide was passed through the filtrate, and the mixture concentrated. The residue was extracted with boiling ethanol from which the sugar slowly crystallised in a desiccator ($CaCl_2$). 2-*O*-Methyl-α-D-mannose (IV) (0·8 g.) (from ethanol) had m. p. 137°, $[\alpha]_D^{19}$ +15° ⟶ +5° (24 hr., constant) (*c*, 1·3 in H_2O), R_G 0·32 in solvent *a* (Found : C, 42·9; H, 7·2; OMe, 16·3. Calc. for $C_7H_{14}O_6$: C, 43·2; H, 7·2; OMe, 16·0%) {Pacsu and Trister [2] give m. p. 136—137°, $[\alpha]_D$ +7·0° ⟶ +4·5° (H_2O)}. The phenylhydrazone had m. p. 163—164° (Pacsu and Trister [2] record m. p. 163°) (Found : OMe, 11·3. Calc. for $C_{13}H_{20}O_5N_2$: OMe, 10·9%). The syrupy mixture of methyl pyranosides, prepared by refluxing the sugar with dry methanolic hydrogen chloride, consumed 1·01 mol. of periodate at 35° in 8 hr. (spectrophotometric determination [14] carried out by Mr. R. J. Ferrier).

Methyl 4 : 6-O-*Ethylidene*-α-D-*mannoside* 3-*Nitrate* (V).—Ice-cold fuming nitric acid (1·5 ml.) in acetic anhydride (3·5 ml.) was added to a suspension of methyl 4 : 6-*O*-ethylidene-α-D-mannoside (1·5 g.) in acetic anhydride (3·5 ml.) at 0°. After 5 min. at 0° the mixture was poured into ice-water (50 ml.) and the aqueous layer was decanted from the syrupy 2 : 3-dinitrate, neutralised with sodium carbonate, and extracted with chloroform (5 × 50 ml.). Concentration of the extract and two crystallisations of the residue from light petroleum–chloroform yielded *methyl* 4 : 6-O-*ethylidene*-α-D-*mannoside* 3-*nitrate* (V) (0·6 g.), m. p. 166°

and mixed m. p. 165° (with a sample prepared by Honeyman and Stening,[9] m. p. 165°), $[\alpha]_D^{20}$ +57° (*c*, 0·9 in $CHCl_3$) (Found : C, 40·6; H, 5·7; N, 5·3. $C_9H_{15}O_8N$ requires C, 40·7; H, 5·7; N, 5·3%).

Methyl 4 : 6-O-*Ethylidene*-2-O-*methyl*-α-D-*mannoside* 3-*Nitrate* (VI).—Silver oxide (3 g.) was added portionwise during 5 hr. to a suspension of the 3-nitrate (V) (3 g.) in boiling methyl iodide (12 ml.), and the mixture was refluxed for a further 20 hr. The silver residues were washed with hot methanol. The combined washings and filtrate were concentrated to give a syrup which crystallised from light petroleum to yield *methyl* 4 : 6-O-*ethylidene*-2-O-*methyl*-α-D-*mannoside* 3-*nitrate* (VI) (2·6 g.), m. p. 100—101° and mixed m. p. 101° (with a sample prepared by Honeyman and Stening,[9] m. p. 101—102°), $[\alpha]_D^{21}$ +45° (*c*, 1·1 in $CHCl_3$) (Found : C, 43·6; H, 6·2; N, 4·6; OMe, 22·0. $C_{10}H_{17}O_8N$ requires C, 43·0; H, 6·1; N, 5·0; OMe, 22·0).

Denitration of Methyl 4 : 6-O-*Ethylidene*-2-O-*methyl*-α-D-*mannoside* 3-*Nitrate*.—A mixture of iron and zinc dust was added portionwise to methyl 4 : 6-*O*-ethylidene-2-*O*-methyl-α-D-mannoside 3-nitrate (2·5 g.) in acetic acid (50 ml.) at 45°. When reaction started the solution was cooled and kept at room temperature for 15 min. (negative diphenylamine–sulphuric acid test). The filtrate and chloroform washings were combined and washed with water, and the chloroform layer was dried (K_2CO_3) and concentrated. The resulting syrup afforded methyl 4 : 6-*O*-ethylidene-2-*O*-methyl-α-D-mannoside (III) (1·9 g.) (from ether–light petroleum), m. p. and mixed m. p. 78—79°, $[\alpha]_D^{20}$ +42° (*c*, 1·2 in $CHCl_3$) (Found : C, 51·1; H, 7·4; OMe, 26·5. Calc. for $C_{10}H_{18}O_6$: C, 51·3; H, 7·7; OMe, 26·3%).

Methyl 4 : 6-O-*Ethylidene*-α-D-*mannoside* 3-*Nitrate* 2-*Toluene*-p-*sulphonate* (VII).—Toluene-*p*-sulphonyl chloride (5·5 g.) in pyridine (10 ml.) was added to a solution of the 3-nitrate (V) (5 g.) in pyridine (10 ml.) at 0°, and the solution kept for 5 days at room temperature. Water was added and, after standing overnight, the solution was extracted with chloroform. The extract was washed with dilute sulphuric acid, and concentrated to a syrup which yielded *methyl* 4 : 6-O-*ethylidene*-α-D-*mannoside* 3-*nitrate* 2-*toluene*-p-*sulphonate* (VII) (5·7 g.) (from methanol), m. p. 123—124° and mixed m. p. 121—123° (with a sample prepared by Honeyman and Stening,[9] m. p. 121—122°), $[\alpha]_D^{19}$ −12° (*c*, 0·8 in $CHCl_3$) (Found : C, 45·9; H, 4·7; N, 3·1. $C_{16}H_{21}O_{10}NS$ requires C, 45·8; H, 5·0; N, 2·8%).

Methyl 4 : 6-O-*Ethylidene*-α-D-*mannoside* 2-*Toluene*-p-*sulphonate* (VIII).—A mixture of iron and zinc dust was added portionwise to a solution of the 3-nitrate 2-toluene-*p*-sulphonate (VII) (5·5 g.) in acetic acid (110 ml.) and after 15 min. (negative diphenylamine–sulphuric acid test) the solid was filtered off and washed with chloroform. The combined filtrate and washings were washed with water, and the chloroform layer was dried (K_2CO_3) and evaporated to a syrup which afforded *methyl* 4 : 6-O-*ethylidene*-α-D-*mannoside* 2-*toluene*-p-*sulphonate* (VIII) (4·2 g.) (from methanol), m. p. 165—166°, $[\alpha]_D^{17}$ +10° (*c*, 1·5 in $CHCl_3$) (Found : C, 50·8; H, 5·9; S, 8·0. $C_{16}H_{22}O_8S$ requires C, 51·3; H, 5·9; S, 8·5%).

Methyl 4 : 6-O-*Ethylidene*-3-O-*methyl*-α-D-*mannoside* 2-*Toluene*-p-*sulphonate* (IX).—Silver oxide (4 × 3 g.) was added to a suspension of the 2-toluene-*p*-sulphonate (VIII) (4 g.) in boiling methyl iodide (15 ml.) during 24 hr., and heating was continued for a further 16 hr. The silver residues were filtered off and washed with chloroform; evaporation gave a syrup which yielded *methyl* 4 : 6-O-*ethylidene*-3-O-*methyl*-α-D-*mannoside* 2-*toluene*-p-*sulphonate* (IX) (3·9 g.) (from light petroleum), m. p. 131°, $[\alpha]_D^{21}$ −16° (*c*, 0·8 in $CHCl_3$) (Found : C, 52·4; H, 6·4; OMe, 8·1. $C_{17}H_{24}O_8S$ requires C, 52·5; H, 6·2; OMe, 8·0%).

3-O-*Methyl*-α-D-*mannose* (X).—Sodium amalgam (4%; 20 g.) was added to methyl 4 : 6-*O*-ethylidene-3-*O*-methyl-α-D-mannoside 2-toluene-*p*-sulphonate (1·7 g.) in methanol–water (9 : 1; 35 ml.), and the mixture was stirred at 45° for 4 hr. and at room temperature for 20 hr. After the solution had been decanted from mercury it was neutralised with carbon dioxide, inorganic salts were filtered off, and the filtrate was evaporated. The residue was extracted with chloroform, and concentration yielded a syrup (0·8 g.; presumably methyl 4 : 6-*O*-ethylidene-3-*O*-methyl-α-D-mannoside). This was heated with 0·5N-hydrochloric acid (15 ml.) at 100° for 5·5 hr. (constant rotation), and the solution was neutralised with silver carbonate. The filtrate was treated with Amberlite resin IR-120(H) and concentrated to a syrup which crystallised from methanol to give 3-O-*methyl*-α-D-*mannose* (X) (0·35 g.), m. p. 133—134° (from ethanol–ether), $[\alpha]_D^{19}$ +14° ⟶ +3° (±1°) (24 hr., constant) (*c*, 0·6 in H_2O) (Found : C, 43·2; H, 6·5; OMe, 16·1. $C_7H_{14}O_6$ requires C, 43·2; H, 7·2; OMe, 16·0%). A mixture with 2-*O*-methyl-α-D-mannose had m. p. 110—125°. The sugar had a similar R_G 0·30 in solvent

a to the 2-methyl ether, but travelled more slowly ($R_{mannose}$ 1·9) than the 2-methyl ether ($R_{mannose}$ 2·1) in solvent *b*. When heated with phenylhydrazine acetate the sugar (X) gave 3-*O*-methyl-D-glucosazone, m. p. 165—166° which gave an *X*-ray powder photograph identical with that given by an authentic sample. The derived syrupy mixture of methyl pyranosides (prepared by refluxing the sugar with dry methanolic hydrogen chloride) consumed 0·0 mol. of sodium metaperiodate solution during 8 hr. at 35°.

Preparation of 1 : 6-*Anhydro*-4-O-*methyl*-β-D-*mannopyranose*.—Finely ground ivory nuts (400 g.) were pyrolysed as described by Knauf, Hann, and Hudson.[15] The dark distillate was neutralised with barium carbonate and treated with charcoal–Celite. Evaporation yielded a thick syrup which was dissolved in acetone (500 ml.) and shaken with concentrated sulphuric acid (3 ml.) for 48 hr. After neutralisation with cupric carbonate the filtrate was concentrated to a syrup which crystallised on addition of propan-2-ol, and after recrystallisation from the same solvent gave 1 : 6-anhydro-2 : 3-*O*-*iso*propylidene-β-D-mannopyranose (20 g.), m. p. 161—162°, $[\alpha]_D^{19}$ −57° (*c*, 1·2 in H_2O).

Methylation acording to the method of Knauf *et al.*[15] gave a product which was dissolved in benzene and chromatographed on alumina (*b*). Elution with ether–light petroleum (b. p. 40—60°) afforded a syrup which crystallised from the same solvent to give 1 : 6-anhydro-4-*O*-methyl-2 : 3-*O*-*iso*propylidene-β-D-mannopyranose (16 g.), m. p. 52—53°, $[\alpha]_D^{19}$ −33° (*c*, 0·7 in $CHCl_3$).

Hydrolysis of the *iso*propylidene compound gave syrupy 1 : 6-anhydro-4-*O*-methyl-β-D-mannopyranose (Found : OMe, 18·0. Calc. for $C_7H_{12}O_5$: OMe, 17·6%).

1 : 6-*Anhydro*-4-O-*methyl*-β-D-*mannopyranose* 2-*Toluene*-p-*sulphonate* (XI).—Toluene-*p*-sulphonyl chloride (4·6 g.) in pyridine (25 ml.) was added to 1 : 6-anhydro-4-*O*-methyl-β-D-mannopyranose (4 g.) in pyridine (25 ml.) at 0°. The mixture was kept for 48 hr. at −5° and then for 24 hr. at 0°. The solution was poured into sodium hydrogen carbonate solution, the mixture evaporated to remove most of the pyridine, the residue extracted with chloroform, and the extract washed with dilute sulphuric acid, sodium hydrogen carbonate solution, and water. Concentration gave a syrup which when crystallised from acetone–ether had m. p. 84—86°. This crystalline substance (5·1 g.) was dissolved in benzene and chromatographed on alumina (*b*). Elution with benzene–ether afforded 1 : 6-*anhydro*-4-O-(*ethyl*-β-D-*mannopyranose* 2-*toluene*-p-*sulphonate* (XI), m. p. 85—87°, $[\alpha]_D^{20}$ −42° (*c*, 1·0 in $CHCl_3$) (Found : C, 51·0; H, 5·4; S, 9·4; OMe, 9·0. $C_{14}H_{18}O_7S$ requires C, 50·9; H, 5·5; S, 9·7; OMe, 9·4%); elution with ether and acetone–ether yielded further small quantities of the same compound but no other substance could be detected.

1 : 6-*Anhydro*-3 : 4-*di*-O-*methyl*-β-D-*mannopyranose* 2-*Toluene*-p-*sulphonate* (XII).—The 2-toluene-*p*-sulphonate (XI) (4 g.) was dissolved in acetone (8 ml.), and methyl iodide (8 ml.) and anhydrous calcium sulphate (5 g.) were added. Silver oxide (3 × 5 g.) was added during 3 hr. to the boiling solution and heating was continued for a further 13 hr. The solid was filtered off and extracted with hot acetone; evaporation gave a syrup which crystallised from acetone–ether. This product (3·5 g.; m. p. 96—100°) was dissolved in benzene and chromatographed on alumina (*b*). Elution with benzene–light petroleum gave a syrup which crystallised from ether to yield 1 : 6-*anhydro*-3 : 4-*di*-O-*methyl*-β-D-*mannopyranose* 2-*toluene*-p-*sulphonate* (XII) (1·8 g.), m. p. 88—89°, $[\alpha]_D^{19}$ −35° (*c*, 1·0 in $CHCl_3$) (Found : C, 52·4; H, 5·5; S, 9·2; OMe, 18·1. $C_{15}H_{20}O_7S$ requires C, 52·3; H, 5·9; S, 9·3; OMe, 18·0%). Elution with ether and acetone–ether gave unchanged starting material (XI) (1·7 g.), m. p. and mixed m. p. 85—87° (from ether); this material was suspended in boiling methyl iodide (5 ml.) and silver oxide (7 g.) was added during 12 hr. The product was worked up in the usual way and chromatographed on alumina (*b*) to give more of the dimethyl ether (XII) (1·4 g.), m. p. and mixed m. p. 87—89°.

1 : 6-*Anhydro*-β-D-*mannopyranose* 2-*Toluene*-p-*sulphonate* (XIII).—1 : 6-Anhydro-β-D-mannopyranose (prepared by the hydrolysis of 1 : 6-anhydro-2 : 3-*O*-*iso*propylidene-β-D-mannopyranose) (3 g.) was dissolved in pyridine (20 ml.) at 0° and a solution of toluene-*p*-sulphonyl chloride (2·5 g.) in pyridine (20 ml.) at 0° was added. The mixture was kept at −5° for 48 hr. and then at 0° for 24 hr. Water (2 ml.) was added and the mixture was poured into water (50 ml.) containing sodium hydrogen carbonate (3 g.). The aqueous layer was decanted from the small quantity of syrup which separated and the solution was concentrated (more syrup separated during the evaporation). The residue was extracted with acetone and the extract concentrated; addition of water gave 1 : 6-*anhydro*-β-D-*mannopyranose* 2-*toluene*-p-

sulphonate (XIII) (2·9 g.), m. p. 146—147° (after recrystallisation from acetone–ether), $[\alpha]_D^{19}$ −74° (*c*, 0·5 in $COMe_2$) (Found : C, 49·3; H, 5·2. $C_{13}H_{16}O_7S$ requires C, 49·4; H, 5·1%).

Methylation of 1 : 6-*Anhydro*-β-D-*mannopyranose* 2-*Toluene*-p-*sulphonate*.—1 : 6-Anhydro-β-D-mannopyranose 2-toluene-*p*-sulphonate (2·5 g.) was dissolved in acetone (5 ml.), and methyl iodide (8 ml.) and anhydrous calcium sulphate (4 g.) were added. Silver oxide (5 × 3 g.) was added during 5 hr. to the boiling solution and heating was continued for a further 7 hr. After being worked up in the usual way the syrup was dissolved in benzene and chromatographed on alumina (*b*). Elution with benzene–light petroleum and crystallisation from ether gave the 3 : 4-dimethyl ether (XII) (1·1 g.), m. p. and mixed m. p. 87—88°. Elution with benzene–ether and crystallisation from ether gave the 4-methyl ether (XI) (1·0 g.), m. p. and mixed m. p. 85—87°. Remethylation of the 4-methyl ether (XI) with methyl iodide and silver oxide followed by chromatography on alumina (*b*) afforded the 3 : 4-dimethyl ether (XII) (0·8 g.), m. p. and mixed m. p. 86—87°.

1 : 6-*Anhydro*-3 : 4-*di*-O-*methyl*-β-D-*mannopyranose* (XIV).—1 : 6-Anhydro-3 : 4-di-*O*-methyl-β-D-mannopyranose 2-toluene-*p*-sulphonate (1·8 g.) was dissolved in methanol–water (9 : 1; 35 ml.), and sodium amalgam (4%; 35 g.) was added. The mixture was stirred at 40° for 4 hr. and at room temperature overnight. The solution and methanol washings were decanted from the mercury and neutralised with carbon dioxide; inorganic salts were filtered off and the filtrate was concentrated. The residue was extracted with chloroform, and the extract evaporated to a syrup which was crystallised from acetone–ether and then from light petroleum–ether. 1 : 6-*Anhydro*-3 : 4-*di*-O-*methyl*-β-D-*mannopyranose* (XIV) (0·85 g.) had m. p. 63—65°, $[\alpha]_D^{20}$ −90° (*c*, 0·6 in $CHCl_3$) (Found : C, 50·7; H, 7·2; OMe, 31·8. $C_8H_{14}O_5$ requires C, 50·5; H, 7·4; OMe, 32·6%).

3 : 4-*Di*-O-*methyl*-D-*mannose* (XV).—1 : 6-Anhydro-3 : 4-di-*O*-methyl-β-D-mannopyranose (0·8 g.) was heated with N-hydrochloric acid (30 ml.) at 100° for 110 min. (constant rotation), and the solution was neutralised with silver carbonate and filtered. Hydrogen sulphide was passed through the solution, the mixture was taken to dryness, and the residue extracted with methanol yielding a solution from which 3 : 4-di-*O*-methyl-α-D-mannose monohydrate, m. p. 109—111°, crystallised. The sugar slowly changed to a more stable crystalline form, m. p. 78—80°. After recrystallisation from acetone–ether the sugar had m. p. 78—80° and mixed m. p. 78—81° (with an authentic sample, m. p. 80—82°), $[\alpha]_D^{19}$ +18° (4 min.) ⟶ +6° (24 hr., constant) (Found : C, 42·2; H, 8·0; OMe, 27·2. Calc. for $C_8H_{18}O_7$: C, 42·5; H, 8·0; OMe, 27·2%). The sugar had R_g 0·67 in solvent *a*, and on oxidation with bromine gave 3 : 4-di-*O*-methyl-D-mannonolactone, m. p. 159—160° and mixed m. p. 159—161° (with an authentic sample, m. p. 161—162°), $[\alpha]_D^{19}$ +178° (20 min.) ⟶ +132° (68 hr., constant) (*c*, 0·56 in H_2O).

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THE SYNTHESIS OF 2-O-β-D-XYLOPYRANOSYL-L-ARABINOSE

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2-*O*-β-D-Xylopyranosyl-L-arabinose, which had $[\alpha]_D$ +47·0°→+32·5°, was first isolated from the products of partial acid hydrolysis of corn cob hemicellulose B.[1] We have recently isolated the same disaccharide, having $[\alpha]_D$ +53·7°→+33·2°, from the partial acid hydrolysis of barley husk and esparto hemicelluloses.[2] It was suggested[1] that the positive rotation of the disaccharide indicated the presence of an α-D-xylopyranosyl linkage. Approximate calculations of the expected optical rotations of *O*-D-xylopyranosyl-L-arabopyranoses may be made using Hudson's rules,[3] and the results are shown in the Table. Although it is not possible to take account of relatively small differences in optical rotation arising from linkages through different possible positions in the arabinose residue, it is clear from these calculations that the observed rotation of the disaccharide is consistent only with the presence of a β-D-xylopyranosyl linkage. Furthermore, comparison with the observed rotation of 3-*O*-α-D-xylopyranosyl-L-arabinose, isolated from the partial acid hydrolysis of golden apple gum[4] and of corn fibre hemicellulose,[5] indicates that the two disaccharides contain different configurations at the glycosidic linkage.

Disaccharide	$[\alpha]_D$
O-α-D-Xylopyranosyl-α-L-arabopyranose	+131° (calc.)
O-α-D-Xylopyranosyl-β-L-arabopyranose	+191° (calc.)
O-β-D-Xylopyranosyl-α-L-arabopyranose	+2° (calc.)
O-β-D-Xylopyranosyl-β-L-arabopyranose	+63° (calc.)
3-*O*-α-D-Xylopyranosyl-L-arabinose	+173° (obs.)[4]
3-*O*-α-D-Xylopyranosyl-L-arabinose	+166°→+181·8° (obs.)[5]

We now report confirmation of the structure of the disaccharide as 2-*O*-β-D-xylopyranosyl-L-arabinose by an unambiguous synthesis. Condensation of 2 : 3 : 4-tri-*O*-acetyl-α-D-xylopyranosyl bromide with a hydroxylic compound results in the formation of the corresponding β-D-xylopyranoside. A suitable hydroxylic compound for this synthesis, benzyl 3 : 4-*O*-*iso*propylidene-β-L-arabopyranoside, was prepared by the condensation of benzyl β-L-arabopyranoside with acetone. Condensation of this compound with tri-*O*-acetyl-α-D-xylopyranosyl bromide under Koenigs-Knorr conditions[6] afforded benzyl 3 : 4-*O*-*iso*propylidene-2-*O*-(2 : 3 : 4-tri-*O*-acetyl-β-D-xylopyranosyl)-β-L-arabopyranoside. Catalytic deacetylation of this compound with sodium methoxide, followed by hydrogenation over palladium/calcium carbonate to cleave the benzyl glycoside and mild acid hydrolysis to remove the *iso*propylidene group, gave the disaccharide and small amounts of xylose and arabinose. After chromatographic separation 2-*O*-β-D-xylopyranosyl-L-arabinose was isolated as the crystalline trihydrate, which was identical (m.p. and mixed m.p., optical rotation, and *X*-ray single crystal photograph) with the disaccharide previously isolated from barley husk and esparto hemicelluloses.[2] It is hoped that full details of this synthesis will be published elsewhere in due course.

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A SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF PERIODATE CONSUMED DURING THE OXIDATION OF CARBOHYDRATES

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It has been shown that the reaction of compounds with periodate in aqueous solution may be followed spectrophotometrically[1] by measuring the decrease in light absorption due to the periodate ion at the maximum of 222·5mμ.[2] We have shown that this method, suitably modified to include an important correction for the light absorption of the iodate ion produced during reaction, is applicable to the determination of periodate consumed by as little as 2–3 mg. of water-soluble carbohydrates.

Determinations are conveniently carried out on quantities of carbohydrate calculated from the formula, $\mu M \times P \simeq 70–100$, where μM is the number of micro-mol. of carbohydrate and P is the number of mol. of periodate likely to be consumed per mol. of substance; suitable quantities are 6–9 mg. of a monosaccharide derivative consuming 2 mol. of periodate or 12–18 mg. of a similar substance consuming 1 mol. Samples were dissolved in 0·015M sodium metaperiodate solution (10 ml.) and the solutions were incubated in the dark at 35°. Aliquots were withdrawn with an " Agla " syringe and diluted 250 times. The optical densities of the resulting solutions were measured in the " Unicam " SP. 500 spectrophotometer at 223 mμ, and compared with those of the original solution of periodate (diluted 250 times) and of an equimolecular iodate solution (optical densities, *ca* 0·6 and 0·1 respectively). When necessary, the initial volume of solution may be reduced and the course of reaction with periodate may be followed using a total of only 2–3 mg. of the carbohydrate.

It has been shown that no correction need be applied for the absorption at the chosen wave-length of either the carbohydrate or its oxidation product. Solutions of the carbohydrates examined all had optical densities of $<0·005$. When excess sugar was added and reaction allowed to proceed to completion, the optical density of the resulting solution was that of pure iodate solution. The absorption of a 0·015M sodium metaperiodate solution does not change over a period of 48 hours when the solution is kept in the dark at 35°.

Experiments were carried out with a wide variety of monosaccharides, hexitols, and methyl glycosides, and in each case the theoretical quantity of periodate was consumed after 8 hours. If the oxidation was prolonged for a further 12 hours a small over-consumption (*ca* 4%) of periodate was observed. In the case of more complex carbohydrates reaction with periodate does not always proceed so simply, but the results obtained by the spectrophotometric method are in good agreement with those obtained by the usual volumetric method[3] (Tables 1 and 2). The oxidation of disaccharides proceeds rapidly in the initial stages but subsequent oxidation takes place slowly, being dependent on the rate of hydrolysis of formyl esters formed as intermediates. Various methyl ethers of monosaccharides react very slowly with periodate and in this respect our results are in agreement with previous observations.[4]

Table I

Carbohydrate oxidized	*Mol. periodate consumed (S)* 8 *hr.*	24 *hr.*	*Mol. periodate consumed (V)* 8 *hr.*	24 *hr.*
Maltose	4·1	4·8	3·8	4·9
Cellobiose	3·8	4·0	4·0	4·2
4-*O*-β-D-mannopyranosyl-D-mannopyranose	3·9	5·1	4·0	4·9
Melibiose	5·8	5·9	5·7	5·8
2 : 3-Di-*O*-methyl-D-xylose	0·28	0·55	0·29	0·56
2 : 4-Di-*O*-methyl-D-galactose	0·32	0·81	0·40	0·83

S = spectrophotometric determination
V = volumetric determination

Satisfactory results were also obtained with a number of polysaccharides, which either were readily soluble in water or could be easily dispersed in hot water.

Table II

	Oat starch[5]		*Inulin*[6]		*Perennial rye grass fructosan*[7]		*Beechwood hemi-cellulose A*[8]	
Time	*Mol. periodate consumed/sugar residue*							
(hours)	(S)	(V)	(S)	(V)	(S)	(V)	(S)	(V)
2·5	0·72	0·70	0·57	0·65	0·99	1·00	0·21	0·18
4·5	0·83	0·85	0·66	0·72	0·99	1·01	—	—
9·5	0·91	0·88	0·80	0·84	1·00	1·04	0·31	0·36
24	0·98	1·00	0·91	0·90	1·07	1·12	0·54	0·57
48	—	—	1·06	1·02	1·17	1·14	0·73	0·76

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THE STRUCTURE OF CALLOSE FROM THE GRAPE VINE

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The term callose is used to denote a group of membranous substances found predominantly in the phloem of plants and characterized by an affinity for the blue dyes of the triphenylmethane series. A typical example is the material which accumulates on the sieve plates of the phloem of the grape vine (*Vitis vinifera*) during winter dormancy and in the early stages of phloem senility. A method for the isolation of small amounts of this substance in pure form has been devised by one of us (G. K.). The lengthy procedure, which involves mechanical separations of phloem cell debris and chemical dissolution of lignin and cellulosic material associated with the callose accumulations, may be conveniently followed under the microscope where the callose particles (*ca.* $30\times10\mu$) are easily seen, and by these means about 140 mg. of the substance was made available for chemical investigation.

Callose, which is insoluble in dilute acids, dilute alkali, and in cuprammonium, had $[\alpha]_D+31°$ in anhydrous formic acid (cf. laminarin, $[\alpha]_D+16°$ in formic acid). Chromatographic examination of the hydrolysate from the polysaccharide showed only glucose, but the presence of *ca.* 2% of uronic acid residues in callose was shown by Kaye and Kent's method.[1] This small proportion of acidic groups may account for the affinity for basic dyes. When a suspension of callose in sodium metaperiodate solution was shaken in the dark, spectrophotometric determination of periodate consumed[2] indicated an uptake of 0·05 mol. per glucose residue after 15 days (in a parallel experiment starch granules consumed 1·05 mol. per glucose residue). This result is consistent only with the presence in the polysaccharide of chains of 1 : 3-linked glucose residues. Conclusive evidence for this mode of linkage came from the isolation of 2 : 4 : 6-tri-*O*-methyl-D-glucose (identified by m.p., mixed m.p., and *X*-ray powder photograph) as the major product (*ca.* 90%) of hydrolysis of the derived methylated polysaccharide. Small quantities of di-*O*-methyl-glucoses (from incomplete methylation) and a methylated uronic acid were also detected chromatographically.

It is clear from these experiments that callose from the grape vine is structurally similar to laminarin[3] in containing chains of 1 : 3-linked β-D-glucopyranose residues. Supporting evidence came from the chromatographic detection of glucose, laminaribiose, and laminaritriose as products of partial acid hydrolysis, and of glucose and laminaribiose as products of enzymic hydrolysis by an emulsin preparation. Full details of this investigation will be published elsewhere.

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840. *The Constitution of Barley Husk Hemicellulose.*

By G. O. Aspinall and R. J. Ferrier.

Barley husk hemicellulose, containing a small proportion (*ca.* 4%) of glucuronic acid residues, gave on hydrolysis xylose and arabinose in the ratio of 6 : 1. 2-*O*-D-Xylopyranosyl-L-arabinose was identified amongst the products of mild acid hydrolysis. Hydrolysis of the methylated polysaccharide afforded 2 : 3 : 5-tri-*O*-methyl-L-arabinose, 3 : 5-di-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, and 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methylglucuronosyl)-xylose in the approximate molar ratio 1 : 1 : 2 : 14 : 3 : 1. It is concluded from these and other experiments that the polysaccharide is composed of chains of 1 : 4-linked β-D-xylopyranose residues to which are attached side-chains of L-arabofuranose and 2-*O*-D-xylopyranosyl-L-arabofuranose residues through position 3, and glucopyranuronic acid residues through position 2. A small degree of branching in the backbone of D-xylose residues is indicated.

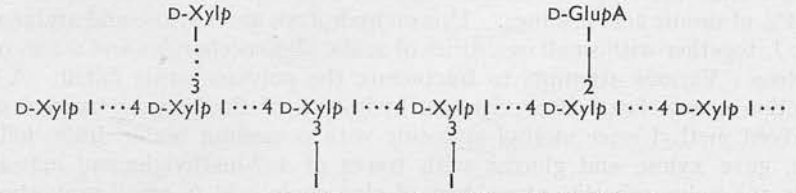
Most lignified tissues contain polysaccharides composed mainly of D-xylose residues. The xylans from various land plants, so far examined, are similar in possessing backbones of 1 : 4-linked β-D-xylopyranose units, but differ in the nature and number of sugar units attached as side-chains.[1] In continuation of structural investigations of the polysaccharide components of barley carried out in these laboratories [2] we have now examined the hemicellulose of barley husk.

Barley husks, separated from the grain, were extracted with benzene–ethanol to remove fats and colouring materials and with hot water to remove adhering starch. Extraction of the husks with cold aqueous sodium hydroxide afforded a hemicellulose containing about *ca.* 4% of uronic acid residues. This on hydrolysis gave xylose and arabinose in the ratio of 6 : 1, together with small quantities of acidic oligosaccharides and traces of glucose and galactose. Various attempts to fractionate the polysaccharide failed. A resistant acidic fraction was isolated after vigorous hydrolysis of the polysaccharide; reduction of the derived methyl ester methyl glycoside with potassium borohydride, followed by hydrolysis, gave xylose and glucose with traces of 4-*O*-methylglucose, indicating the presence in the polysaccharide of residues of glucuronic acid (a small proportion as the 4-methyl ether).

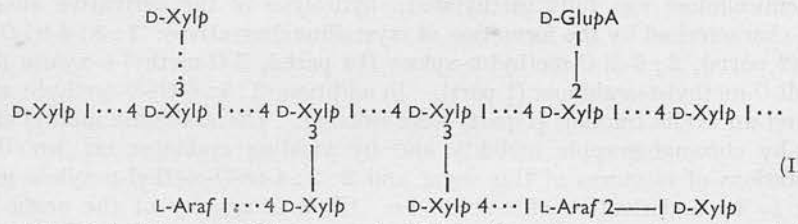
The hemicellulose was fully methylated; hydrolysis of the derivative afforded the following, characterised by the formation of crystalline derivatives: 2 : 3 : 4-tri-*O*-methyl-D-xylose (2 parts), 2 : 3-di-*O*-methyl-D-xylose (14 parts), 2-*O*-methyl-D-xylose (3 parts), and 3 : 5-di-*O*-methyl-L-arabinose (1 part). In addition, 2 : 3 : 5-tri-*O*-methyl-L-arabinose (1 part) and an acidic fraction (1 part) were isolated. The arabinose methyl ether was identified by chromatographic mobility and by yielding arabinose on demethylation, optical rotations of mixtures of this sugar and 2 : 3 : 4-tri-*O*-methyl-D-xylose indicating the sugar to be a derivative of L-arabinose. Chromatography of the acidic fraction showed the presence of at least two components, the minor one travelling on the chromatogram at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. The acidic fraction was converted into the methyl ester methyl glycoside, reduction of which followed by hydrolysis gave 2 : 3 : 4-tri-*O*-methylglucose and 3-*O*-methylxylose in the ratio of 1·2 : 1 together with a trace of 2 : 3-di-*O*-methylxylose. A portion of the reduced acidic fraction was remethylated and after hydrolysis yielded 2 : 3 : 4 : 6-tetra-*O*-methylglucose and 3 : 4-di-*O*-methylxylose. Although insufficient of the acidic fraction was available for the complete characterisation of its hydrolysis products, these observations show that

the major component was 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methylglucuronosyl)xylose. It follows from the methylation studies that this polysaccharide contains chains of 1 : 4-linked β-D-xylopyranose units to which are attached at least three types of side-chain, glucuronic acid units directly linked to xylose through position 2, side-chains terminated by L-arabofuranose units and linked to the backbone through position 3 of xylose, and side-chains terminated by D-xylopyranose units and linked to the backbone through position 3 of xylose. It is not possible to decide on the present evidence whether the small amount of D-xylose also isolated from the hydrolysis of the methylated polysaccharide is of structural significance, arising from a double-branching point, or whether the sugar results from incomplete methylation of the polysaccharide or demethylation during hydrolysis.

Evidence for the mode of attachment of the non-terminal L-arabofuranose residues follows from the isolation of 2-*O*-D-xylopyranosyl-L-arabinose from the products of mild acid hydrolysis of the polysaccharide. This disaccharide, isolated as the trihydrate, although differing slightly in physical constants from those reported by Whistler and McGilvray,[3] gave an *X*-ray powder photograph identical with that of an authentic sample kindly provided by Dr. D. I. McGilvray. It is probable that the xylopyranose residue of the disaccharide is derived from a non-reducing end-group in the polysaccharide since the disaccharide was released under relatively mild conditions of hydrolysis and no evidence was found for the presence in the hydrolysate of higher oligosaccharides containing arabinose residues. Although it is not possible to advance a unique structure for this hemicellulose, structures (I) and (II) are consistent with the results so far presented. The following evidence, however, shows that at least some terminal L-arabofuranose residues are linked directly to the backbone of D-xylose residues as in structure (I). Hydrolysis of the periodate-oxidised polysaccharide indicated the presence in the polysaccharide of xylose (21 ± 1·5%) and arabinose (6 ± 0·5%) residues unattacked by periodate. Controlled acid hydrolysis of the polysaccharide under mild conditions resulted in selective hydrolysis of some arabofuranosyl linkages with the formation of a degraded poly-



(I)



(II)

[Sugar residues joined by dotted lines may be linked either directly or through a chain of 1 : 4-linked D-xylopyranose residues].

saccharide. It was not possible, however, to remove all the arabinose residues without extensive hydrolysis of xylopyranosyl linkages. Hydrolysis of the periodate-oxidised degraded polysaccharide indicated the presence therein of xylose (16 ± 1·5%) and arabinose (5 ± 0·5%) residues unattacked by periodate. The decrease in xylose residues unattacked by periodate after selective removal of arabofuranose residues would be expected on the basis of structure (I), whereas no such decrease would result from the removal of

arabofuranose residues in structure (II). The proportion of arabinose residues in the degraded polysaccharide, which are unattacked by periodate, indicates that the non-terminal arabofuranosyl linkages are more resistant to acid hydrolysis than terminal arabofuranosyl linkages. Indeed, under the conditions used in the isolation of 2-*O*-D-xylopyranosyl-L-arabinose, the disaccharide was accompanied by appreciable quantities of xylose-containing oligosaccharides. It is not possible, therefore, on the present evidence to decide whether these disaccharide side-chains are also attached directly to the xylan backbone.

A molecular-weight determination by the isothermal-distillation method (by courtesy of Drs. C. T. Greenwood and W. N. Broatch) gave a value of 10,500 $\pm$ 500 (degree of polymerisation, 66 $\pm$ 3) for the methylated polysaccharide. The methylation analysis indicated the presence of some six non-reducing D-xylopyranose end-groups per molecule; three such end-groups, as shown by the isolation of 2-*O*-D-xylopyranosyl-L-arabinose on partial acid hydrolysis of the original polysaccharide and by the quantity of 3 : 5-di-*O*-methyl-L-arabinose formed on hydrolysis of the methylated polysaccharide, must be linked to the non-terminal arabinose residues. The presence in the molecule, therefore, of three non-reducing D-xylopyranose end-groups linked to other xylose residues indicates some branching in the backbone of D-xylose residues. From the quantity of 2-*O*-methyl-D-xylose formed on hydrolysis of the methylated polysaccharide it is clear that these branch points must involve 1 : 3-linkages.

These results show that this barley husk hemicellulose contains many of the structural features encountered in other polysaccharides of the xylan group.[1] Here, as in the wood xylans and as in several xylans isolated from the Gramineae, glucuronic acid (either unsubstituted or as its 4-methyl ether) is found linked directly to position 2 of a xylose residue in the backbone of the molecule. Again, terminal non-reducing L-arabofuranose residues, linked to the main chain through $C_{(3)}$ of the xylose residue, are commonly present in polysaccharides of this group. A novel feature of this xylan is the occurrence of non-terminal L-arabofuranose units found in the 2-*O*-D-xylopyranosyl-L-arabofuranose grouping. This disaccharide has been isolated previously from the partial acid hydrolysis of corn cob hemicellulose B;[3] since cleavage of the disaccharide was effected under mild conditions it is probable that in the latter case also this grouping is attached to the backbone of the molecule through an arabofuranosyl linkage. In the barley husk hemicellulose some branching in the backbone of xylose residues is clearly indicated. In the case of several other polysaccharides of this group, however, definite proof of the presence or absence of such branching must await the development of more precise methods of structural analysis.

In the course of the investigation the husk hemicellulose was isolated in a similar way from the corresponding sample of malted barley. A preliminary examination showed that the polysaccharide was qualitatively similar to that isolated from the original barley; thus the same sugar units were present, and chromatography showed that mild acid hydrolysis released arabinose and 2-*O*-xylopyranosylarabinose. Small quantitative differences were shown between the two polysaccharides, *e.g.*, the hemicellulose from the malted barley gave rather less formic acid on periodate oxidation than the barley hemicellulose. The somewhat higher glucosan content in the malted barley polysaccharide probably arose from incomplete removal of starchy polysaccharides since treatment with salivary α-amylase resulted in the formation of glucose and maltose. It is probable, therefore, that only minor changes have taken place in the barley husk hemicellulose during malting.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (*A*) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer); (*B*) butan-1-ol–ethanol–water (4 : 1 : 5; upper layer); (*C*) benzene–ethanol–water

(169 : 47 : 15; upper layer); (*D*) butan-1-ol–formic acid–water (500 : 115 : 385; upper layer); (*E*) ethyl acetate–pyridine–water (10 : 4 : 3).

Isolation of Barley Husk Hemicellulose.—Barley (Carlsberg variety, harvested in 1953) (10 kg.) in batches (80 g.) was passed through an automatic polishing machine. The husks separated in this manner (*ca.* 10% of the grain) were contaminated with a large amount of starchy powder which was removed by means of a 20 mesh sieve, leaving behind the husk (470 g.) The husks were extracted for 24 hr. with boiling benzene–ethanol (2 : 1) in order to inactivate enzymes and to remove fats and colouring materials. Further extractions with cold water, hot water (75—80°), and with 0·01N-sodium hydroxide (under nitrogen) resulted in complete removal of starch and of water-soluble polysaccharides. The hemicellulose was extracted in the following manner: husks (80 g.) were extracted four times with N-sodium hydroxide (750 ml.), each extraction being carried out for 16 hr. in an atmosphere of nitrogen. The extracts were acidified with glacial acetic acid to pH 4—5 and the polysaccharide was precipitated by addition of acetone (0·8 vol.). The total yield (34 g.) of hemicellulose represented 0·34% by weight of the original barley or 7·2% of the weight of the husk. The polysaccharide had $[\alpha]_D^{16}$ −102° (*c* 0·5 in 0·5N-sodium hydroxide), and chromatographic examination of the hydrolysate by Flood, Hirst, and Jones's [4] method with solvent *A* showed the presence of xylose (67%), arabinose (11%), glucose (2%), and galactose (1%) [Found: ash, 1·2; lignin, 7·8; uronic anhydride (by decarboxylation), 3·7; OMe, 1·2%]. Attempts to fractionate the polysaccharide by precipitation of the copper complex and by precipitation from aqueous solution with ammonium sulphate failed to yield components significantly different in composition.

The polysaccharide (10 g.) was hydrolysed with 2N-sulphuric acid (150 ml.) for 4 hr. at 100°. The solution was neutralised with barium carbonate, the filtrate was concentrated to 50 ml., barium ions were removed by treatment with Amberlite resin IR-100(H), and the solution was concentrated. The resulting syrup was fractionated on acid-washed charcoal–celite (1 : 1, w/w) (60 × 8 cm.), elution with water yielding monosaccharides and elution with ethyl methyl ketone–water (5 : 95, v/v) yielding acidic oligosaccharides together with some neutral sugars (50 mg.). Further fractionation on filter sheets with solvent *A* afforded an acidic oligosaccharide (17 mg.) and a trace of an unidentified sugar. The acidic component was converted into the methyl ester methyl glycoside, reduced with potassium borohydride, and hydrolysed, chromatography showing approximately equal amounts of xylose and glucose, and a trace of 4-*O*-methylglucose.

Methylation of Barley Husk Hemicellulose.—The polysaccharide (10 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide. The product (6·5 g.) was fractionated by precipitation from chloroform with light petroleum (b. p. 60—80°), giving two main fractions: 1, precipitated with 3 vol. of light petroleum {4·2 g.; $[\alpha]_D$ −92° ($CHCl_3$); OMe, 38·7%}, and 2, precipitated with 4 vol. of light petroleum {1·6 g.; $[\alpha]_D$ −95° ($CHCl_3$); OMe, 38·7%}; these fractions were combined and were used in subsequent experiments.

Hydrolysis of Methylated Hemicellulose and Separation of Methylated Sugars.—The methylated polysaccharide (4·4 g.) was hydrolysed successively with boiling methanolic 3% hydrogen chloride (500 ml.) for 2·5 hr. and with 0·5N-hydrochloric acid (500 ml.) at 100° for 4·5 hr. A small quantity (0·12 g.) of insoluble material was separated at this stage; further hydrolysis yielded no detectable sugars [Found: OMe, 31·0; lignin, 80%]. Evaporation after neutralisation with silver carbonate yielded a syrup (4·3 g.), which was treated (in aqueous solution) with barium carbonate (0·2 g.). The mixture of methylated sugars (4·02 g.) was fractionated on cellulose (90 × 3 cm.) by elution with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water, butan-1-ol partly saturated with water, and with water to give nine fractions.

Fraction 1. The sugar (34 mg.) travelled on the chromatogram in solvent *B* at the same rate as 2 : 3 : 5-tri-*O*-methyl-L-arabinose and/or 2 : 3 : 4-tri-*O*-methyl-D-xylose. Demethylation showed only arabinose, and chromatography in solvent *C* showed only tri-*O*-methylarabinose (Found: OMe, 48·2. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). Attempts to prepare crystalline derivatives failed.

Fractions 2 *and* 3. Chromatography showed that both these fractions (79 and 270 mg.) contained mixtures of 2 : 3 : 5-tri-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methylxylose. Calculations from the observed optical rotations, $[\alpha]_D$ −31° and +9° (H_2O), indicated that fractions

2 and 3 contained 86% and 19% respectively, of 2 : 3 : 5-tri-*O*-methyl-L-arabinose {2 : 3 : 5-tri-*O*-methyl-L-arabinose has $[\alpha]_D$ −39·5° (H_2O), and 2 : 3 : 4-tri-*O*-methyl-D-xylose has $[\alpha]_D$ +20° (H_2O)}.

Fraction 4. The sugar (80 mg.) travelled on the chromatogram in solvents *B* and *C* at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-xylose and slowly crystallised (Found: OMe, 48·2. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). After recrystallisation from acetone–light petroleum the sugar had m. p. and mixed m. p. 87—89° and $[\alpha]_D$ +64·5° (2 min.) ⟶ +20° (80 min., const.) (*c* 0·6 in H_2O). The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 100—101°.

Fraction 5. The syrup (141 mg.) had $[\alpha]_D$ −16° (*c* 0·6 in H_2O) (Found: OMe, 35·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%) and on demethylation yielded arabinose. Its rate of movement on the chromatogram in solvent *B* was the same as that of 2 : 5-di-*O*-methyl-L-arabinose, but the brown coloration (and yellow fluorescence in ultraviolet light) with aniline oxalate differed markedly from the grey coloration (and pink fluorescence in ultraviolet light) given by the 2 : 5-isomer. The sugar was further distinguished from the 2 : 5-dimethyl ether by its considerably greater ionophoretic mobility. The sugar was identified as 3 : 5-di-*O*-methyl-L-arabinose by conversion into 3 : 5-di-*O*-methyl-L-arabonolactone, m. p. 68—71°, which gave an *X*-ray powder photograph (by courtesy of Dr. C. A. Beevers) identical with that of an authentic specimen, and into 3 : 5-di-*O*-methyl-L-arabonamide, identified by its m. p. and mixed m. p. 144·5—145° and by its *X*-ray powder photograph.

Fraction 6. The syrup (2·113 g.) crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose and had m. p. and mixed m. p. 81—83° and $[\alpha]_D^{17}$ −20·6° (4 min.) ⟶ +24·9° (65 min., const.) (*c* 1·2 in H_2O) (Found: OMe, 35·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The identity of the sugar was confirmed by its conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 123—124°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 133—134°.

Fraction 7. The syrup (412 mg.) slowly yielded a crystalline sugar together with a small quantity of syrup. The crystalline material was ionophoretically pure 2-*O*-methylxylose; the syrupy material contained mainly the 2-methyl ether with a trace of the 3-methyl ether. Recrystallisation afforded 2-*O*-methyl-β-D-xylose, m. p. and mixed m. p. 133—134°, $[\alpha]_D^{17}$ −5·6° ⟶ +34·4° (90 min., const.) (*c* 1·4 in H_2O) (Found: OMe, 19·1. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). The derived 2-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 125—126°.

Fraction 8. The sugar (34 mg.) had m. p. 140—142° and mixed m. p. (with D-xylose) 141—144°, and gave an *X*-ray powder photograph identical with that of D-xylose.

Fraction 9. The fraction (259 mg.) obtained by elution of the cellulose with water was purified by solution in hot methanol, and after removal of barium ions by treatment with Amberlite resin IR-120(H) yielded a syrup (154 mg.), $[\alpha]_D^{17}$ +96° (H_2O) (Found: OMe, 29·6. Calc. for a tetra-*O*-methylaldobiouronic acid, $C_{15}H_{26}O_{11}$: OMe, 32·4%). Chromatography in solvent *D* showed two components, the minor and faster-moving one travelling at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. On further hydrolysis the slower-moving component decreased in amount with the formation of more tri-*O*-methylglucuronic acid and of a mono-*O*-methylxylose. Some of the acidic fraction (50 mg.) was converted into the methyl ester methyl glycoside by refluxing it with methanolic 1% hydrogen chloride (25 ml.) for 6 hr. The product was dissolved in dry ether (25 ml.), and lithium aluminium hydride (50 mg.) was added slowly during 3 hr. to the boiling solution. Water was added to the cooled solution to destroy excess of hydride, and the mixture was acidified with sulphuric acid and extracted with chloroform. Hydrolysis of the extract with N-sulphuric acid for 5 hr. at 100° yielded two main components *a* and *b* together with a trace of 2 : 3-di-*O*-methylxylose. Quantitative determination showed that fractions *a* and *b* were present in the ratio of 1 : 1·2. Fraction *a* travelled on the chromatogram in solvent *B* at the same rate as 2- and/or 3-*O*-methyl-D-xylose; paper ionophoresis showed that the fraction was mainly 3-*O*-methylxylose with only a trace of the 2-methyl ether. Fraction *b* travelled on the chromatogram at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucose and gave glucose on demethylation.

Another portion of the acidic fraction (30 mg.) was converted into the methyl ester methyl glycoside and reduced with lithium aluminium hydride as described previously. The reduction product was methylated with methyl iodide and silver oxide and the methylated disaccharide was hydrolysed with 2N-hydrochloric acid for 4 hr. at 100° yielding two sugars *c* and *d*. In

solvent *B* sugar *c* travelled on the chromatogram slightly more slowly than 2 : 3-di-*O*-methyl-D-xylose and at a rate corresponding to 3 : 4-di-*O*-methylxylose. The ionophoretic mobility of sugar *c* was considerably greater than that of 2 : 3-di-*O*-methylxylose as would be expected for the 3 : 4-dimethyl ether. Sugar *d* was chromatographically indistinguishable from 2 : 3 : 4 : 6-tetra-*O*-methylglucose; a small sample was obtained crystalline, m. p. 76—84°

Partial Acid Hydrolysis of Hemicellulose and Isolation of 2-O-D-*Xylopyranosyl-*L*-arabinose.*—Barley husk hemicellulose (13 g.) was heated in aqueous 0·02N-oxalic acid (1·3 l.) at 96° for 3·5 hr. The cooled solution was neutralised with calcium carbonate, filtered, concentrated, and poured into ethanol (10 vol.) to precipitate material of high molecular weight. The supernatant liquid was concentrated to a syrup (*ca.* 3 g.) which was fractionated on charcoal–celite (1 : 1, w/w/) (25 × 4 cm.), elution with water giving monosaccharides (1·7 g.), and elution with ethanol–water (5 : 95) giving fractions (i) (0·39 g.) and (ii) (0·145 g.). Fraction (i) contained a major component with R_{xylose} 0·67 in solvent *E* and a minor component with R_{xylose} 0·59, travelling at the same rate as xylobiose. Fraction (ii) contained the same two components (mainly xylobiose) together with traces of slower-moving oligosaccharides having R_{xylose} 0·40, 0·28, and 0·14. The main component from fraction (i) crystallised readily and after several recrystallisations from ethanol–water had m. p. 97—98°, $[\alpha]_D^{19}$ +53·7° (6 min.) ⟶ +33·2° (2 hr., const.) (*c* 1·3 in H_2O) (Found: C, 36·1; H, 7·0. Calc. for $C_{10}H_{18}O_9,3H_2O$: C, 35·7; H, 7·2%). A molecular-weight determination from a single crystal *X*-ray photograph and a density measurement (kindly carried out by Mr. H. W. Erhlich) gave a value of 333 ± 6 (calc. for a disaccharide trihydrate, 336). Hydrolysis of the sugar gave xylose and arabinose, but after oxidation with bromine water hydrolysis gave only xylose. The sugar was chromatographically indistinguishable from and gave an *X*-ray powder photograph identical with that of 2-*O*-D-xylopyranosyl-L-arabinose.

Periodate Oxidation of Hemicellulose.—Hemicellulose (269 mg.) was dissolved in potassium chloride solution (0·56M; 60 ml.), and sodium metaperiodate (0·2M; 20 ml.) was added. The formic acid released, estimated by Anderson, Greenwood, and Hirst's method,[5] reached a constant value of 0·10 mole per pentose residue after 7 days. Oxidation of the polysaccharide with sodium metaperiodate solution indicated a consumption of periodate of 0·85 mole per pentose residue (constant after 2 days).

Estimation of Sugar Residues Unattacked by Periodate.—The polysaccharide (0·5 g.) in sodium metaperiodate solution (0·2M, 25 ml.) was set aside for 54 hr. Periodate and iodate ions were precipitated by the addition of barium chloride solution and the resulting solution was dialysed against distilled water for 3 days; concentration and precipitation with acetone afforded the periodate-oxidised polysaccharide (0·25 g.). Hydrolysis of this material and chromatographic examination of the hydrolysate,[4] using galactose as reference sugar, showed the presence of xylose (21 ± 1·5%) and arabinose (6 ± 0·5%).

The hemicellulose (0·8 g.) was heated with aqueous oxalic acid (0·02N; 80 ml.) at 96° for 1 hr. (these represent optimun conditions for the removal of arabinose residues without extensive degradation of the molecule). The cooled solution was neutralised with calcium carbonate, and the centrifugate was poured into acetone (10 vol.) to precipitate degraded polysaccharide. The mother liquor was concentrated and sugars (0·057 g.) were extracted with boiling methanol. Quantitative chromatography showed the presence in the methanol extract of arabinose and xylose in the ratio of 11 : 1 together with traces of xylosylarabinose and other oligosaccharides. The degraded hemicellulose was converted into the corresponding periodate-oxidised polysaccharide, hydrolysis of which afforded xylose (16 ± 1·5%) and arabinose (5 ± 0·5%) (these figures are expressed as percentages of the original hemicellulose).

Husk Hemicellulose from Malted Barley.—Malted barley (Carlsberg variety, harvested in 1953) (700 g.) was given a rapid treatment in an automatic milling machine, and most of the husks were removed from the fragmented grain particles. The husks (63 g.) were extracted in the manner described previously except that four extractions with hot water for 8 hr. each were required to remove the adhering starch. Alkaline extraction afforded hemicellulose (6·2 g.), which was further extracted with boiling ethanol–water (4 : 1) to ensure complete removal of contaminating sugars. The hemicellulose had $[\alpha]_D$ −81° (*c* 0·5 in 0·5N-NaOH) and gave on hydrolysis xylose, arabinose, and glucose in the ratio of 76 : 16 : 8. Digestion of a 1% solution of the polysaccharide with salivary α-amylase in phosphate–citrate buffer at pH 6·8 at 35° followed by chromatographic examination of the hydrolysate showed that maltose and glucose were formed. Partial acid hydrolysis of the polysaccharide yielded

arabinose and xylopyranosylarabinose, together with traces of xylose and xylose-containing oligosaccharides. A sample of the hemicellulose was oxidised with potassium metaperiodate, and the formic acid released [5] reached a constant value of 0·09 mole per pentose residue after 11 days.

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896. *The Glucomannans from Sitka Spruce* (Picea sitchensis).

By G. O. Aspinall, R. A. Laidlaw, and R. B. Rashbrook.

Hemicellulose fractions composed of D-mannose and D-glucose residues have been isolated from Sitka spruce holocellulose. A methylated glucan and a methylated glucomannan have been prepared therefrom, hydrolysis affording the corresponding 2 : 3 : 6-trimethyl ethers. It is concluded from these and other results that both glucan and glucomannan are linear polysaccharides composed of β-1 : 4-linked sugar residues.

Glucomannans have been isolated from a number of plant sources. The majority of these, for example, the glucomannans from iris seeds [1] and lily bulbs,[2] and the " Iles Mannan " from the tubers of some *Amorphophallus* species,[3,4] appear to function as reserve polysaccharides. All these materials contain essentially linear molecules composed of β-1 : 4-linked sugar residues. Of these polysaccharides, only in the case of " Iles Mannan " has evidence been brought forward to show that glucose and mannose are constituents of the same polysaccharide, rather than arising from a mixture of a glucan and a mannan. It is well known that coniferous woods contain cell-wall polysaccharides giving glucose and mannose on hydrolysis, but little is known concerning the mode or order of linkage of the constituent sugars. Anthis [5] has isolated from the partial hydrolysis of slash pine α-cellulose (containing 10% of mannan) two disaccharides 4-*O*-β-D-glucopyranosyl-α-D-mannose and a mannosylglucose which may be 4-*O*-β-D-mannopyranosyl-α-D-glucose; these disaccharides clearly arise from a glucomannan in the cell-wall structure. It is not clear, however, whether such a polysaccharide is a component of the hemicellulose fraction which has resisted extraction by alkali or whether the glucomannan participates in the highly ordered structure of the cellulose. Recently Jones and his collaborators [6] have fractionated the hemicelluloses of Loblolly pine and isolated a glucomannan which also contains a small proportion of D-galactose residues. Here again an essentially linear structure composed of 1 : 4-linked β-D-glucose and β-D-mannose units is indicated. Partial acid hydrolysis of this polysaccharide afforded the following oligosaccharides, 4-*O*-β-D-glucopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-glucose, and *O*-β-D-mannopyranosyl-(1 ⟶ 4)-*O*-β-D-mannopyranosyl-(1 ⟶ 4)-D-mannose.[7] These results were interpreted as indicating the presence of either a single glucomannan in which glucose and mannose residues are unevenly distributed, or a mixture of a glucomannan and one or more other mannans.

The present paper reports studies carried out on the glucomannans from Sitka spruce (*Picea sitchensis*). Partially delignified sawdust was extracted with cold aqueous alkali to give a complex mixture of polysaccharides yielding glucose, mannose, galactose, xylose, and arabinose on hydrolysis. The residual wood was completely delignified and the resulting holocellulose was extracted with cold 10% sodium hydroxide solution; the hemicellulose fraction after precipitation as the copper complex gave on hydrolysis glucose and mannose in approximately equal amounts. Methylation of the material resulted in the loss of the mannose-containing polysaccharide and a methylated glucan was isolated with an optical rotation indicating a β-linked polymer. Hydrolysis of the methylated polysaccharide gave 2 : 3 : 4 : 6-tetra-*O*-methylglucose (identified by chromatographic mobility and by giving glucose on demethylation), 2 : 3 : 6-tri-*O*-methyl-D-glucose (identified as the crystalline sugar), and some di-*O*-methylhexose (probably arising from incomplete methylation of the polysaccharide). A molecular-weight determination by the isothermal-

distillation method (by courtesy of Dr. C. T. Greenwood) gave a value of 8900 ± 500 (degree of polymerisation, 41—46) for the methylated polysaccharide. Although the quantity of non-reducing end-group isolated (1 in 80) was rather less than that required by a linear polymer, it is clear that this methylated polysaccharide is composed of unbranched chains of 1 : 4-linked β-D-glucopyranose residues and is indistinguishable on the present evidence from a methylated degraded cellulose.

The second sample of glucomannan, prepared in a similar manner, contained a higher proportion of mannose residues (mannose : glucose, 2·5 : 1). The consumption of periodate on oxidation was in excess of 1 mole per sugar unit; it is probable, however, that the over-consumption resulted from reactions other than α-glycol scission since the quantity of formic acid released was insufficient to indicate any large proportion of non-reducing end-groups or of 1 : 6-linked hexose units. The quantity of formic acid released during the oxidation was consistent with that from a linear hexosan of *ca.* 45 units. The polysaccharide was converted into a methylated glucomannan, whose optical rotation indicated a β-linked polymer. Hydrolysis of the methylated polysaccharide afforded a tetra-*O*-methylhexose (chromatographically indistinguishable from the 2 : 3 : 4 : 6-tetramethyl ethers of mannose and/or glucose but shown to be mainly the mannose derivative by optical rotation), a mixture of tri-*O*-methylhexoses, and some di-*O*-methylhexoses probably arising from incomplete methylation of the polysaccharide. Separation of the tri-*O*-methylhexoses was effected by selective methyl furanoside formation,[3] and the 2 : 3 : 6-trimethyl ethers of D-mannose and D-glucose were identified as their respective di-*p*-nitrobenzoates. The proportion of 2 : 3 : 6-tri-*O*-methyl-D-mannose to 2 : 3 : 6-tri-*O*-methyl-D-glucose was estimated as 3 : 1 from the optical rotation of the mixture of sugars in water and by the change of optical rotation (undergone solely by the glucose derivative) in methanolic hydrogen chloride. A molecular-weight determination by the isothermal-distillation method gave a value of 10,000 ± 500 (degree of polymerisation, 47—51) for the methylated polysaccharide. This value taken together with the quantity of non-reducing end-group (1 in *ca.* 35) indicated that the polysaccharide has an essentially linear structure, although the possibility of a small proportion of molecules containing a single branch point cannot at present be excluded.

Partial acid hydrolysis of the glucomannan was effected by treating the polysaccharide with a mixture of acetic anhydride, acetic acid, and concentrated sulphuric acid at 0° and deacetylating the products with barium methoxide. It has been shown previously [5] that no reversion of monosaccharides occurs under these conditions. A preliminary chromatographic and ionophoretic examination of the products of partial acid hydrolysis showed the presence of glucose, mannose, cellobiose, mannobiose, and a mannosylglucose. The isolation of a mannosylglucose indicates that there is present in the spruce hemicellulose fraction a polysaccharide composed of both mannose and glucose residues.

These results indicate that the glucomannan fraction of Sitka spruce hemicellulose contains at least two essentially linear components, a β-1 : 4-linked glucan and a β-1 : 4-linked glucomannan. On the available evidence we cannot exclude the possibility also of a polysaccharide composed solely of mannose residues. The glucomannan component is clearly similar to the glucomannans previously examined from other plant sources.[1-4] It is increasingly evident from these results and from those of other workers [5-7] that in the coniferous woods there is no clear dividing line on grounds of solubility or of chemical structure between the ordered cellulosic framework, usually of high molecular weight, and the less highly ordered cell-wall polysaccharides or hemicelluloses, usually of lower molecular weight. In the present study it has been shown that polysaccharides composed solely of glucose units, and apparently differing from normal cellulose only in molecular size, may be extracted from the wood, together with glucomannan, by alkali. On the other hand, it is not yet clear whether the mannose units present in some cellulose preparations [5,8] are part of the true cellulose or arise from mannan or glucomannan occluded in

the cellulose. It is noteworthy in this respect that the alkaline extraction of synthetic mixtures of ramie cellulose and ivory-nut mannan, prepared by denitration of a mixture of the nitrated polysaccharides, failed to remove all the mannan.[9] Further, evidence for the presence of mannose units in cellulose nitrates of high molecular weight [8] does not adequately prove the presence of mannose units in cellulose since fractional precipitation of mixtures of the nitrates of high-molecular-weight mannose-free ramie cellulose and relatively low-molecular-weight ivory-nut mannan afforded fractions of high molecular weight containing mannose units.[9]

Experimental

Paper chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (C) benzene–ethanol–water (170 : 50 : 15, upper layer). Paper ionophoresis was carried out in borate buffer at pH 10.[10]

Extraction of Sitka Spruce Glucomannan.—Air-dried sawdust (290 g.), which had been previously extracted under reflux with ethanol–water (85 : 15) and with benzene, was extracted successively with cold and hot water; small quantities of polysaccharide giving on hydrolysis galactose, glucose, and mannose, together with traces of arabinose and xylose, were isolated. Partial removal of lignin was effected by treatment of the residue with sodium chlorite solution acidified by the addition of acetic acid (procedure of Chanda, Hirst, Jones, and Percival [11]), yielding a spruce holocellulose (194 g., dry wt.) (Found: lignin, 5·5%). This material was extracted twice with cold 4% aqueous sodium hydroxide, and neutralisation of the extract with acetic acid followed by the addition of two volumes of ethanol afforded a polysaccharide fraction (9 g.) which gave on hydrolysis glucose, galactose, mannose, arabinose, and xylose. Further extraction of the wood with cold 24% aqueous potassium hydroxide gave a similar complex mixture of hemicelluloses (11 g.). The residual wood was washed with water and delignified again to give a lignin-free residue (105 g., dry wt.). This material was extracted four times with 10% aqueous sodium hydroxide (2 l.) and the extracts were each treated with Fehling's solution (200 ml.). The gelatinous blue precipitates were combined and decomposed with acetic acid, and the resulting solid was washed with 80% acetic acid to remove copper salts and with acetone to remove acid and yielded a white powder. This material was twice precipitated from alkaline solution as the copper complex and regenerated to give glucomannan (sample A; 9 g.). Hydrolysis of the polysaccharide gave glucose and mannose in approximately equal quantities. Sample B, used in all the later experiments, was isolated in a similar manner except that the final reprecipitations *via* the copper complex were omitted. Sample B had $[\alpha]_D^{18}$ −33° (*c* 1·1 in 2N-NaOH) and chromatography of the hydrolysate showed the presence of mannose and glucose in the ratio of 2·5 : 1, together with a trace of xylose.

Methylation of Sample A.—Glucomannan (7·4 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide; neutralisation of the mixture followed by dialysis to remove inorganic salts gave partially methylated polysaccharide (5 g.) (OMe, 38·7%). After a further series of treatments with methyl sulphate and sodium hydroxide the methoxyl content of the methylated polysaccharide (3·5 g.) was raised to 41·6%. A further methylation with methyl iodide and silver oxide failed to raise the methoxyl content of the methylated polysaccharide (3·1 g.) beyond 42% {$[\alpha]_D^{20}$ −9·4° (*c* 3·5 in $CHCl_3$)} {cf. methylated cellulose $[\alpha]_D$ −4° (in $CHCl_3$) [12]}. The methylated glucomannan (1·7 g.) was hydrolysed by formic acid (40 ml.) at 100° for 5 hr.; after removal of formic acid the residual syrup was heated with N-hydrochloric acid (50 ml.) at 100° for 3 hr. After neutralisation with silver carbonate the hydrolysate was concentrated to a syrup (1·6 g.). The mixture of methylated sugars was fractionated on cellulose, with light petroleum (b. p. 100—120°)–butan-1-ol (6 : 4) saturated with water as eluant to give three fractions. Fraction 1 (18 mg.) travelled on the chromatogram at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and gave glucose on demethylation. Fraction 2 (1·32 g.) crystallised and gave only glucose on demethylation. After two recrystallisations from ether–light petroleum (b. p. 60—80°) the sugar had m. p. and mixed m. p. (with 2 : 3 : 6-tri-*O*-methyl-D-glucose) 115—117°, $[\alpha]_D^{17}$ +100·5° (initial) ⟶ +70·6° (*c* 1·47 in H_2O), and $[\alpha]_D^{17}$ +58·5° ⟶ −36° (28 hr., constant) (*c* 1·49 in methanolic hydrogen chloride). Fraction 3 (93 mg.) contained a mixture of di-*O*-methylhexoses which were not examined further.

Periodate Oxidation of Glucomannan.—Samples of the glucomannan (*ca.* 30 mg.) were shaken

in the dark with 0·3M-sodium metaperiodate solution (12 ml.), and determination of the periodate consumed gave the following results (moles consumed per $C_6H_{10}O_5$ residue) : 1·11 (48 hr.); 1·24 (117 hr.); 1·34 (261 hr.).

The glucomannan was oxidised with potassium metaperiodate solution, and the formic acid liberated was determined. The following results were obtained (expressed as the number of $C_6H_{10}O_5$ residues per mole of formic acid liberated); 18·0 (125 hr.); 15·1 (177 hr.); 13·5 (243 hr.); 12·4 (310 hr.). As the formic acid liberated did not reach a constant value, extrapolation to zero time gave a value corresponding to formic acid released from α-glycol scission, namely, 1 mole per 15 $C_6H_{10}O_5$ residues. The release of two mols. of formic acid from the reducing end-group and one mol. from the non-reducing end-group being assumed, this value corresponded to a chain length of 45 residues.

Methylation of Glucomannan.—The glucomannan (sample B) (2·5 g.) was converted into its thallium derivative which was heated with methyl iodide; [13] the product was treated three times in a similar manner and then methylated with silver oxide and methyl iodide, to give a methylated glucomannan (0·77 g.) (Found: OMe, 42·0%), whose methoxyl content could not be raised on further methylation $\{[\alpha]_D^{18} -13°$ (*c* 2·48 in $CHCl_3)\}$.

The methylated polysaccharide (0·65 g.) was heated with formic acid (20 ml.) in a sealed tube at 100° for 5 hr.; after removal of formic acid the residual syrup was heated with N-hydrochloric acid (25 ml.) at 100° for 3 hr., neutralised with silver carbonate, and concentrated to a syrup (0·54 g.). The mixture of methylated sugars was fractionated on cellulose (53 × 2·1 cm.), with butan-2-one saturated with water as eluant, to give four fractions.

Fraction 1 (15 mg.) had $[\alpha]_D^{18}$ +8° (*c* 1·8 in H_2O) and was chromatographically indistinguishable from the 2 : 3 : 4 : 6-tetramethyl ethers of D-mannose and D-glucose in solvents B and C.

Fraction 2 (367 mg.) travelled on the chromatogram at the same rate as 2 : 3 : 6-tri-*O*-methyl-D-mannose and -D-glucose. The optical rotation in water $\{[\alpha]_D$ +10·2° (*c* 3·5)} corresponded to that of a mixture of 2 : 3 : 6-tri-*O*-methyl-D-mannose (74%) and 2 : 3 : 6-tri-*O*-methyl-D-glucose (26%). The change in optical rotation in methanolic 1% hydrogen chloride $\{[\alpha]_D^{17}$ +22° ⟶ −4° (*c* 0·49)} indicated the presence in the mixture of 25% of 2 : 3 : 6-tri-*O*-methyl-D-glucose. A portion (67 mg.) was dissolved in methanolic 1% hydrogen chloride and kept at room temperature for 20 hr. $\{[\alpha]_D^{17}$ +22° ⟶ − 4° (constant)}. After neutralisation with silver carbonate and concentration the derived syrup was fractionated on cellulose (40 × 1·4 cm.) with butan-2-one saturated with water as eluant, to give fraction *a* (23 mg., non-reducing) and fraction *b* (41 mg., reducing). Fraction *a* was hydrolysed by heating with 0·1N-sulphuric acid at 100° for 2 hr., and the resulting reducing syrup (19 mg.) was identified as 2 : 3 : 6-tri-*O*-methyl-D-glucose by conversion into the di-*p*-nitrobenzoate, m. p. and mixed m. p. 187—189·5°. The 2 : 3 : 6-tri-*O*-methyl-D-mannose in fraction *b* was identified by conversion into the di-*p*-nitrobenzoate, m. p. and mixed m. p. (with sample m. p. 187—188°) 181·5—185° (a mixture of the di-*p*-nitrobenzoates of D-glucose and D-mannose trimethyl ethers had m. p. 167—173°). Fractions 3 (92 mg.; R_G 0·55 in solvent B) and 4 (20 mg.; R_G 0·37 in solvent B) contained mixtures of di- and mono-*O*-methyl-sugars which were not examined further.

Acetolysis of Glucomannan and Examination of Derived Oligosaccharides.—The glucomannan (1·0 g.) was dissolved in a mixture of acetic anhydride (12 ml.), acetic acid (12 ml.), and concentrated sulphuric acid (1·2 ml.) at 0° and set aside at room temperature for 120 hr. The mixture was poured into ice-water, neutralised with sodium hydrogen carbonate, and extracted with chloroform, and the extract was dried and concentrated to a syrup (1·0 g.). N-Methanolic barium methoxide (1 ml.) was added to a solution of the sugar acetates in methanol (25 ml.). The mixture was shaken at room temperature for 1 hr. and exactly neutralised with sulphuric acid, and the filtrate was concentrated to a syrup (360 mg.). Chromatography showed the presence of mannose, glucose, traces of galactose and xylose, two disaccharides ($R_{mannose}$ 0·52 and 0·38 in solvent A) and traces of other oligosaccharides. Samples of the two disaccharide components were isolated by fractionation on filter sheets, with solvent A. Component A gave a single spot on the chromatogram ($R_{mannose}$ 0·52) and gave mannose and glucose on hydrolysis, but paper ionophoresis showed two components to be present which had the same mobilities as cellobiose and 4-*O*-β-D-mannopyranosyl-D-mannose. Component B was chromatographically ($R_{mannose}$ 0·38) and ionophoretically identical with 4-*O*-β-D-mannopyranosyl-D-glucose and had $[\alpha]_D^{20}$ +9° (*c* 2·3 in H_2O). Hydrolysis afforded glucose and mannose whereas hydrolysis of the derived glycitol gave only mannose.

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900. *Cereal Gums. Part II.* The Constitution of an Araboxylan from Rye Flour.*

By G. O. Aspinall and R. J. Sturgeon.

A water-soluble polysaccharide isolated from rye flour gave on hydrolysis xylose (60%), arabinose (29%), and glucose (5%). From hydrolysis of the methylated polysaccharide, controlled acid hydrolysis of the polysaccharide, and quantitative estimation of xylose residues unattacked by periodate in the original and degraded polysaccharides it is concluded that this highly-branched araboxylan contains chains of 1 : 4-linked β-D-xylopyranose residues with approximately every second xylose residue carrying a terminal L-arabofuranose residue linked through position 3.

The water-soluble gums isolated from cereal grains contain residues of D-glucose, D-xylose, and L-arabinose in varying proportions. Hexosan-rich and pentosan-rich fractions may be obtained either by graded precipitation from aqueous solution by ammonium sulphate [1,2] or by fractional precipitation of the derived acetylated polysaccharides.[3,4] The β-glucans from barley [5] and oats [6] are similar to lichenin [7] in structure in containing chains of 1 : 3- and 1 : 4-linked D-glucopyranose residues. The pentosan from wheat flour has been studied by Perlin [3] and by Montgomery and Smith.[4] The L-arabinose residues are present exclusively as non-reducing end-groups in the furanose form, and it is probable that these units are directly attached to a backbone of 1 : 4-linked β-D-xylopyranose residues. It has been shown by Preece and Hobkirk [2] that the main component of the water-soluble gum fraction from rye flour is an araboxylan of similar composition to the polysaccharide from wheat flour. We are very grateful to Professor I. A. Preece for kindly placing at our disposal a quantity of the rye araboxylan for structural investigation, the results of which are described in this paper.

The polysaccharide had a high negative rotation ($[\alpha]_D$ −107° in N-NaOH) and yielded on hydrolysis xylose (60%), arabinose (29%), and glucose (5·5%). Hydrolysis of the derived methylated polysaccharide afforded the following sugars, characterised by crystalline derivatives: 2 : 3 : 5-tri-*O*-methyl-L-arabinose (30%), 2 : 3-di-*O*-methyl-D-xylose (36%), 2-*O*-methyl-D-xylose (31%), and D-xylose (2·5%). In addition, chromatography showed traces of 2 : 3 : 4-tri-*O*-methylxylose, tri-*O*-methylglucose, and 3-*O*-methylxylose. These results indicate the presence in the polysaccharide of chains of 1 : 4-linked D-xylose residues with branching mainly through position 3. All the side-chains are terminated by L-arabofuranose residues, this being the sole mode of linkage of the arabinose residues. It is not certain whether the small amount of D-xylose isolated from the hydrolysis of the methylated polysaccharide represents some double branching points or whether the sugar arises from incomplete methylation of the polysaccharide or demethylation during hydrolysis. It is probable that the glucose residues present in the polysaccharide and the tri-*O*-methylglucose isolated on hydrolysis of the methylated polysaccharide arise from a contaminating glucan since no methyl ethers of glucose could be detected in the hydrolysate of another fraction of methylated polysaccharide.

On the basis of the methylation results two probable structures (I and II) may be put forward for the repeating unit of the polysaccharide. The following results provide evidence in favour of structure (I). Hydrolysis of the periodate-oxidised polysaccharide indicated the presence in the polysaccharide of xylose (24—25%) residues unattacked by periodate. This value is slightly lower than would be expected if all the arabinose residues were attached to singly branched xylose residues (*ca.* 29%). Controlled hydrolysis of the polysaccharide caused selective cleavage of some of the arabofuranosyl linkages with the

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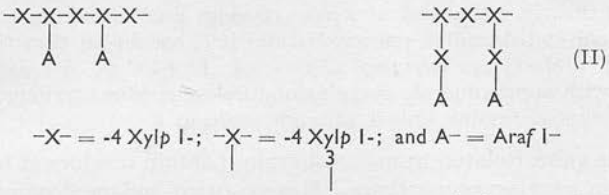
formation of a degraded polysaccharide, giving on hydrolysis xylose (60%) and arabinose (10%) (these and subsequent values are expressed as percentages of the undegraded polysaccharide). Hydrolysis of the periodate-oxidised degraded polysaccharide indicated the presence therein of xylose (8%) residues unattacked by periodate. The reduction (*ca.* 16—17%) in xylose residues unattacked by periodate accompanying the controlled degradation of the polysaccharide corresponds approximately to the decrease (*ca.* 19%) in arabinose residues. This result would be expected on the basis of structure (I) only, and shows that the majority, at least, of the L-arabofuranosyl residues must be attached directly to position 3 of β-D-xylopyranose residues present in the essentially linear backbone of the molecule.



−X− = -4 Xylp l-; −X− = -4 Xylp l-; and A− = Araf l−
3

The araboxylan from rye flour is in many respects similar to the araboxylan from wheat flour,[3,4] notably in that the L-arabofuranose units, present only as end-groups, are attached directly to the backbone of 1 : 4-linked β-D-xylopyranose units. The main structural difference between the two polysaccharides lies in the mode of attachment of some of the arabofuranose residues. In the rye pentosan the majority at least of the arabinose residues are linked through $C_{(3)}$ of singly branched xylose residues, whereas in the wheat pentosan an appreciable proportion of arabinose residues are also linked through $C_{(2)}$ of doubly branched xylose residues. It is noteworthy that terminal L-arabofuranose units linked to $C_{(3)}$ of 1 : 4-linked β-D-xylopyranose units are commonly found in xylans from lignified tissues, especially of the *Gramineae*.[8]

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer); (B) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (C) benzene–ethanol–water (169 : 47 : 15, upper layer).

Rye araboxylan had $[\alpha]_D^{18}$ −107° (*c* 1·2 in N-NaOH); a sample was hydrolysed with N-sulphuric acid for 4 hr. at 100°, and chromatographic examination [9] of the hydrolysate in solvent A then showed the presence of xylose (60%), arabinose (29%), and glucose (5·5%).

Methylation of Rye Araboxylan.—The polysaccharide (2 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide. The product (1·8 g.) was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 60—80°), to give a main fraction (1·33 g.), soluble in chloroform–light petroleum (3 : 7), which had $[\alpha]_D^{18}$ −113° (*c* 0·5 in $CHCl_3$) and was used in subsequent experiments (Found: OMe, 38·8%). Chromatography of the hydrolysate in solvent B showed tri-, di-, and mono-*O*-methylpentoses, together with smaller quantities of xylose and tri-*O*-methylglucose. A minor fraction (0·28 g.) of the methylated polysaccharide, soluble in chloroform–light petroleum (4 : 6), had $[\alpha]_D^{18}$ −121° (*c* 0·5 in $CHCl_3$), and chromatography of the hydrolysate showed the same pentose derivatives but no tri-*O*-methylglucose.

Hydrolysis of Methylated Araboxylan and Separation of Methylated Sugars.—The methylated polysaccharide (1·3 g.) was kept in N-hydrochloric acid (250 ml.) at 30° for 6 days, and the solution was then heated at 100° for 6 hr. After neutralisation with silver carbonate, concentration gave a syrupy mixture of sugars. The syrup (1·06 g.) was fractionated on cellulose (60 × 3 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water as eluant to give five fractions.

Fraction 1. The syrup (273 mg.) had $[\alpha]_D^{18}$ −36·3° (*c* 0·45 in H_2O) (Found: OMe, 46·4. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). Chromatography in solvent C showed 2 : 3 : 5-tri-*O*-methylarabinose and a trace of 2 : 3 : 4-tri-*O*-methylxylose, and demethylation gave arabinose and a

trace of xylose. The major component was identified as 2 : 3 : 5-tri-*O*-methyl-L-arabinose by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 130—132°.

Fraction 2. The syrup (105 mg.) had $[\alpha]_D^{18}$ +24·2° (*c* 0·44 in H_2O) (Found: OMe, 35·5. Calc. for $C_7H_{14}O_5$: OMe, 34·8%), and chromatography in solvent B showed 2 : 3-di-*O*-methylxylose and a small amount of tri-*O*-methylglucose. The syrup crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose and had m. p. and mixed m. p. 76—78°. The derived 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 121—122°. Approximate calculation from optical rotation indicated the presence in the fraction of 102 mg. of di-*O*-methyl-D-xylose and 3 mg. of tri-*O*-methyl-D-glucose.

Fraction 3. The chromatographically pure sugar (199 mg.) crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose, and had m. p. and mixed m. p. 80—81° and $[\alpha]_D^{18}$ −20·1° ⟶ +22·6° (equil.) (*c* 0·35 in H_2O) (Found: OMe, 34·7. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The aniline derivative had m. p. and mixed m. p. 121—123°.

Fraction 4. The crystalline sugar (237 mg.), after recrystallisation from methanol–water, had m. p. and mixed m. p. (with 2-*O*-methyl-β-D-xylose) 130°, and $[\alpha]_D^{18}$ −9·5° ⟶ +35° (equil.) (*c* 0·75 in H_2O) (Found: OMe, 18·7. Calc. for $C_6H_{12}O_5$: OMe, 18·8%). Ionophoretic examination of the mother-liquors showed that a small amount of the 3-methyl ether was also present.

Fraction 5. The syrup (17 mg.) travelled on the chromatogram at the same rate as D-xylose, had $[\alpha]_D^{18}$ +18° (*c* 0·75 in H_2O), and was characterised by conversion into the di-*O*-benzylidene dimethyl acetal, m. p. and mixed m. p. 208—209°.

Estimation of Sugar Residues Unattacked by Periodate.—The polysaccharide (352 mg.) was dissolved in water (10 ml.), sodium metaperiodate (792 mg.) was added, and the solution set aside in the dark for 4 days. Excess of barium chloride solution was added, insoluble barium salts were filtered off, and the filtrate was dialysed for 3 days. Concentration of the solution to small volume and addition of acetone (10 vol.) precipitated the periodate-oxidised polysaccharide (190 mg.). Hydrolysis of this material with N-sulphuric acid for 4 hr. at 100° and chromatographic examination [9] of the hydrolysate, using galactose as reference sugar, showed the presence of xylose (24%).

The polysaccharide (502 mg.) was dissolved in 0·01N-oxalic acid (50 ml.) and heated on the boiling-water bath for 1·5 hr. Ethanol (5 vol.) was added to the cooled solution, and degraded polysaccharide (387 mg.) was precipitated. Chromatography of the supernatant liquor showed only arabinose. Hydrolysis of the degraded polysaccharide afforded xylose (60%) and arabinose (10%) (these and the subsequent value are expressed as percentages of the undegraded polysaccharide). The degraded polysaccharide was converted into the corresponding periodate-oxidised polysaccharide, hydrolysis of which with N-sulphuric acid for 4 hr. at 100°, followed by chromatography [9] of the hydrolysate, showed xylose (8%).

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46. *The Mannans of Ivory Nut* (Phytelephas macrocarpa). *Part II.*[1] *The Partial Acid Hydrolysis of Mannans A and B.*

By G. O. Aspinall, R. B. Rashbrook, and (in part) G. Kessler.

Partial acetolysis of mannan A, followed by deacetylation, afforded a homologous series of β-1 : 4-linked oligosaccharides (mannobiose to mannopentaose). Smaller quantities of mannose-containing oligosaccharides with some α-1 : 4-linkages, and of β-1 : 4-linked oligosaccharides containing both mannose and glucose residues were isolated. A similar series of oligosaccharides was isolated from mannan B. The significance of these results is discussed.

It was shown in Part I [1] that ivory nut mannans A and B are both mixtures of at least two molecular species, terminated by D-mannopyranose and D-galactopyranose residues; both species are linear, but in addition to the dominant β-1 : 4-mannopyranosyl linkages, the evidence indicated the presence also of some mannose residues linked through $C_{(1)}$ and $C_{(6)}$. In order to obtain further information concerning the detailed molecular structure of these polysaccharides we have studied the products of partial acid hydrolysis.

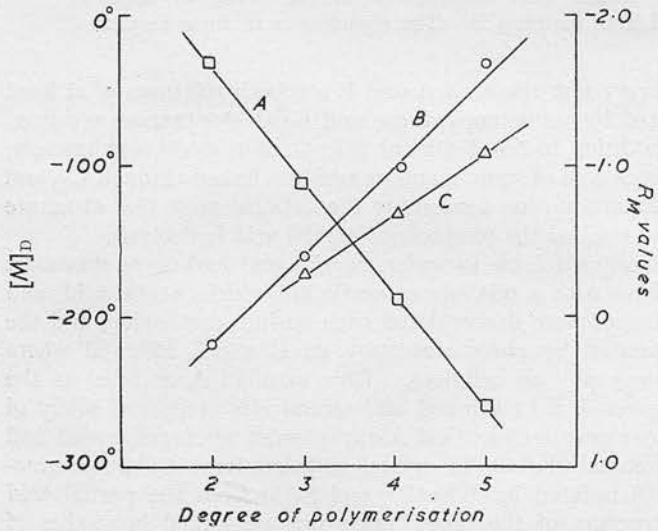
Since the mannans are relatively insoluble in water, partial acid hydrolysis was most conveniently effected by acetolysis with a mixture of acetic anhydride, acetic acid, and sulphuric acid; the products thereof were deacetylated with barium methoxide, and the resulting mixtures were fractionated by chromatography on charcoal, followed where necessary by partition chromatography on cellulose. Thus mannan A afforded as the main products a homologous series of β-1 : 4-linked oligosaccharides composed solely of D-mannose residues. Mannobiose gave an identical *X*-ray powder photograph and had a similar melting point, but differed slightly in optical rotation from 4-*O*-β-D-mannopyranosyl-D-mannose (I; $n = 0$) isolated by Whistler and Stein from the partial acid hydrolysis of guaran.[2] The structure of the disaccharide was confirmed in studies of methylation and periodate oxidation. Mannotriose crystallised as the trihydrate and gave an identical *X*-ray powder photograph and had a similar melting point, but again differed slightly in optical rotation from the β-1 : 4-linked mannotriose (I; $n = 1$) previously isolated from the partial acid hydrolysis of guaran.[3] The derived mannotri-itol dodeca-acetate had similar physical constants to those quoted by Whistler and Smith.[3] Mannotetraose (I; $n = 2$) and mannopentaose (I; $n = 3$) were established as members of the same homologous series by the following observations: (i) the molecular rotations and the R_M values [4] of the sugars, mannobiose to mannopentaose, when plotted against degree of polymerisation gave straight line graphs (see Figure); (ii) chromatographic examination of the products of partial acid hydrolysis showed mannose and the lower homologues of the series.

(I) Man*p* 1$\overset{\beta}{-}$[—4 Man*p* 1$\overset{\beta}{-}$]$_n$—4 Man*p* Man*p* 1$\overset{\alpha}{-}$[—4 Man*p* 1$\overset{\beta}{-}$]$_n$—4 Man*p* (II)

Man*p* 1$\overset{\beta}{-}$[—4 Man*p* 1$\overset{\beta}{-}$]$_n$—4 Glu*p* (III)

Small quantities of two other series of oligosaccharides were also isolated; the first series contained only mannose residues, but in the second series each oligosaccharide contained mannose residues and one glucose residue per molecule. Disaccharide A gave

only mannose on hydrolysis and had an optical rotation indicative of an α-glycosidic linkage. The following observations leave little doubt that this disaccharide is 4-*O*-α-D-mannopyranosyl-D-mannose (II; $n = 0$): (i) periodate oxidation [5] afforded formaldehyde, indicating the absence of a 1 : 5- or 1 : 6-linkage; (ii) reaction with phenylhydrazine gave a disaccharide phenylosazone, showing that a 1 : 2-linkage was not present; (iii) chromatography showed the presence of a tetrose (erythrose) when the disaccharide was oxidised with lead tetra-acetate under controlled conditions and the product hydrolysed,[6] indicating the presence of a 1 : 4-linked disaccharide (under similar conditions 1 : 3-linked hexose-containing disaccharides yield pentoses). Confirmation of the presence of the α-1 : 4-linkage in the disaccharide came from experiments carried out on trisaccharide C. Hydrolysis of the crystalline trisaccharide gave only mannose, and partial acid hydrolysis gave both mannobioses and mannose, but partial acid hydrolysis of the derived glycitol (potassium borohydride reduction) gave only the α-linked mannobiose and mannose. When oxidised with periodate the quantity of reagent consumed by the trisaccharide was exactly parallel to the quantity consumed by the β-linked mannotriose (I; $n = 1$), suggesting the



Molecular rotations and R_M *values of mannose-containing oligosaccharides plotted against degree of polymerisation.*

A, $[M]_D$ *values of ligosaccharides* (I). *B,* R_M *values of oligosaccharides* (I). *C,* R_M *values of oligosaccharides* (II).

presence of similar linkages in the two trisaccharides. The optical rotation of trisaccharide C was consistent with the presence of one α-linked and one β-linked D-mannopyranosyl residue. Proof that the non-reducing end-group (with the α-configuration) was glycosidically linked to position 4 of the central mannose unit in the trisaccharide came from the isolation of 2 : 3 : 4 : 6-tetra- and 2 : 3 : 6-tri-*O*-methyl-D-mannose from the hydrolysis of the methylated glycitol. Trisaccharide C is, therefore, *O*-α-D-mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-D-mannopyranose (II; $n = 1$). Small quantities of a second tetrasaccharide and a second pentasaccharide were also isolated. It is probable from chromatographic examination of the oligosaccharides and their partial hydrolysis products that these belong to the same homologous series (II; $n = 2$ and 3).

The glucose-containing oligosaccharides were not isolated in sufficient quantity for complete characterisations to be carried out. The following observations, however, suggest that the disaccharide B is probably 4-*O*-β-D-mannopyranosyl-D-glucose (III; $n = 0$): (i) hydrolysis of the disaccharide gave glucose and mannose, but hydrolysis of the derived aldobionic acid (bromine oxidation) gave only mannose; (ii) the optical rotation indicated that the mannosylglucose was β-linked; (iii) periodate oxidation [5] afforded formaldehyde, indicating the absence of a 1 : 5- or 1 : 6-linkage; (iv) reaction with phenylhydrazine gave a disaccharide phenylosazone, showing that a 1 : 2-linkage

was not present; (v) controlled oxidation of the disaccharide with lead tetra-acetate followed by hydrolysis of the oxidation product gave erythrose, pointing to a 1 : 4-linkage. Since the phenylosazone of disaccharide B was different from that derived from β-1 : 4-linked mannobiose (I; $n = 0$), there is still doubt concerning the structure of the disaccharide. Trisaccharide D, which gave on hydrolysis mannose and glucose in the approximate ratio of 2 : 1, had an optical rotation indicative of β-linkages. Partial acid hydrolysis of the trisaccharide gave mannose, glucose, mannobiose (I; $n = 0$), and disaccharide A, but on hydrolysis of the derived glycitol only mannose and mannobiose could be detected. Subject to the same reservations as in the case of disaccharide B, the trisaccharide probably belongs to the same homologous series (III; $n = 1$).

The partial acid hydrolysis of mannan B afforded a similar series of oligosaccharides. Again, the major products were the β-1 : 4-linked oligosaccharides (I; $n = 0$, 1, and 2), which were isolated as the crystalline sugars. The other oligosaccharides (II; $n = 0$ and 1) and (III; $n = 0$ and 1) which had been isolated from mannan A were identified chromatographically.

These results provide additional evidence for the presence in the ivory nut mannans of chains of 1 : 4-linked β-D-mannopyranosyl units, and again show that there are no essential structural differences between mannans A and B. The presence, however, of a small proportion of α-linked D-mannose units was not previously suspected. From the present evidence it is probable that the α-linked oligosaccharides are of structural significance and are not artefacts, since control experiments provided no evidence for their formation either from mannose by reversion or from the β-linked oligosaccharides by acid-catalysed anomerisation at the glycosidic bond.[7] It is not yet possible to assess the structural significance of the glucose-containing oligosaccharides. Although the ivory nut mannans contain a small proportion of glucose residues so that partial hydrolysis should give rise to some glucose-containing oligosaccharides, these substances may have been formed by epimerisation of the mannose-containing oligosaccharides. No further evidence was found to confirm the presence in the mannans of 1 : 6-linkages.[1]

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (C) butan-2-one saturated with water; (D) ethyl acetate–propan-2-ol–water (16 : 10 : 5).

Mannan A was prepared by extraction of delignified ivory nut shavings with 7% aqueous potassium hydroxide as described previously.[1] The polysaccharide, which had $[\alpha]_D^{18}$ −48° (*c* 1·1 in N-NaOH), was used without further purification. Mannan B was prepared as described previously [1] and had $[\alpha]_D^{18}$ −26° (*c* 1·0 in anhydrous formic acid).

Acetolysis of Mannan A and Fractionation of Derived Oligosaccharides.—Mannan A (55 g.) was added slowly with stirring to a mixture of acetic anhydride (330 ml.), glacial acetic acid (330 ml.), and concentrated sulphuric acid (33 ml.) at 0°. The mixture was kept at room temperature for 72 hr., during which the mannan had completely dissolved (*ca.* 36 hr.). The mixture was filtered, then poured slowly with stirring into ice-water, and sodium hydrogen carbonate was added gradually (to pH 3—4). The precipitated sugar acetates were filtered off, and the filtrate was extracted with chloroform (3 × 800 ml.). The solid acetates were dissolved in chloroform, the solution was combined with the chloroform extracts, and the combined solutions were washed with sodium hydrogen carbonate solution, dried, and concentrated. A solution of barium methoxide (7·5 g.) in methanol (75 ml.) was added to a solution of the sugar acetates (90·7 g.) in chloroform (250 ml.) and methanol (500 ml.) at 0°, and the mixture was shaken for 1 hr. and set aside overnight at 0°. The mixture was exactly neutralised by the addition of dilute sulphuric acid, and water (175 ml.) was added. The chloroform layer was separated, and the aqueous layer was filtered through a pad of " Celite " and concentrated. The mixture of sugars (45·8 g.), dissolved in water (200 ml.), was added to a column of charcoal–Celite (1·3 kg.; 2 : 1). Elution with water and water containing 0·5—4·0% of ethanol yielded a mixture of monosaccharides (mannose, together with small quantities of xylose, glucose, and

galactose) (6·5 g.) which was not examined further. Ologosaccharides were eluted with ethanol–water containing increasing proportions of ethanol and eight fractions were collected.

Examination of Ologosaccharide-containing Fractions.—Fraction 1. The sugar (9·8 g.; eluted with water containing 4·5—6·5% of ethanol) was crystallised from ethanol–water and had $R_{mannose}$ 0·52 in solvent A, m. p. 202—203°, and $[\alpha]_D^{19}$ −5·2° ⟶ −8·5° (2 hr., const.) (*c* 5·4 in H_2O). Whistler and Stein [2] report m. p. 193·5—194° and $[\alpha]_D^{25}$ −7·7° ⟶ −2·2° (equil.) (in H_2O) for 4-*O*-β-D-mannopyranosyl-D-mannopyranose, but the two samples gave identical *X*-ray powder photographs (comparison kindly undertaken by Professor R. L. Whistler). Treatment of a sample of the sugar with sodium metaperiodate solution [8] resulted in the consumption of 4·0 mol. of reagent after 48 hr., followed the consumption of a fifth mol. after 120 hr.; further reaction occurred slowly thereafter. On reaction with potassium metaperiodate [8] 3 mol. of formic acid were released after 185 hr., but no definite break in reaction occurred at this point. A sample (*ca.* 1 g.) of the sugar was methylated successively with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, to yield the fully methylated disaccharide (310 mg.). A portion of the methylated sugar (100 mg.) was hydrolysed by N-sulphuric acid in a sealed tube at 100° for 6 hr. After neutralisation with barium carbonate, the hydrolysate was separated on filter sheets (Whatman 3MM), with solvent B, to give fractions *a* (35 mg.; R_G 1·0) and *b* (25 mg.; R_G 0·80). These sugars were identified as 2 : 3 : 4 : 6-tetra- and 2 : 3 : 6-tri-*O*-methyl-D-mannose by conversion into their respective aniline derivatives, m. p. and mixed m. p. 141—143° and 120·5—123°. On reaction with phenylhydrazine 4-*O*-β-D-mannopyranosyl-D-mannose gave a phenylosazone, which crystallised in spherulitic aggregates of needles and had m. p. 203—206° (Found: *M*, 526, by absorption [9] at 396 mμ).

Fraction 2. The syrup (1·28 g.; eluted with water containing 6·5—8·0% of ethanol) contained three components, 4-*O*-β-D-mannopyranosyl-D-mannose, and disaccharides A and B, having $R_{mannose}$ 0·52, 0·62, and 0·38. Chromatographically pure samples of disaccharides were obtained by successive fractionations on columns of powdered cellulose and on filter sheets with solvent D.

Disaccharide A had $[\alpha]_D^{17}$ +49° (*c* 0·6 in H_2O) and gave only mannose on hydrolysis. Periodate oxidation of the disaccharide in sodium hydrogen carbonate buffer [5] afforded formaldehyde, identified as the dimedon derivative, m. p. and mixed m. p. 187—190° (under similar conditions no formaldehyde was formed from gentiobiose). A sample of the disaccharide (*ca.* 1 mg.) was dissolved in water (0·01 ml.) and glacial acetic acid (0·09 ml.), and lead tetra-acetate (5 mg.) in glacial acetic acid (0·4 ml.) was added. After 2 hr. excess of oxalic acid was added, and the solution was diluted with water and heated on the water-bath with Amberlite resin IR-120(H). Chromatographic examination of the hydrolysate showed erythrose but no arabinose (under similar conditions laminaribiose afforded arabinose). On reaction with phenylhydrazine the disaccharide gave a phenylosazone, m. p. 200—201° (Found: *M*, 600, by absorption [9] at 396 mμ).

Disaccharide B had $[\alpha]_D^{17}$ +5·5° (*c* 3·5 in H_2O) and gave mannose and glucose on hydrolysis. Hydrolysis of the derived aldonic acid gave only mannose. Periodate oxidation of the disaccharide in sodium hydrogen carbonate buffer [5] afforded formaldehyde, identified as the dimedon derivative, m. p. and mixed m. p. 187—190°. Lead tetra-acetate oxidation of the disaccharide followed by chromatographic examination of the hydrolysate gave erythrose but no arabinose. On reaction with phenylhydrazine the disaccharide gave a phenylosazone, m. p. 149—152° (Found: *M*, 542, by absorption at 396 mμ), which crystallised in leaflets and gave an *X*-ray powder photograph different from that of the phenylosazone from 4-*O*-β-D-mannopyranosyl-D-mannose.

Fraction 3. The sugar (5·6 g.; eluted with water containing 9·0—10·0% of ethanol) was crystallised from ethanol–water and had $R_{mannose}$ 0·22 in solvent A, m. p. (rapid heating) 134·5—135·5°, and $[\alpha]_D^{17}$ −15·7° ⟶ −20·2° (equil.) (*c* 1·29 in H_2O) (Found: C, 39·0; H, 7·1. Calc. for $C_{18}H_{32}O_{16},3H_2O$: C, 38·7; H, 6·9%). Whistler and Smith report m. p. 137—137·5° and $[\alpha]_D^{25}$ −24·7° ⟶ −23·3° for *O*-β-D-mannopyranosyl-(1 ⟶ 4)-*O*-β-D-mannopyranosyl-(1 ⟶ 4)-D-mannopyranose trihydrate, but the two samples gave identical *X*-ray powder photographs (comparison kindly undertaken by Professor R. L. Whistler). On slow heating on the Kofler hot-stage the sugar had m. p. 166·5—169·5° (presumably that of the anhydrous form); Dr. L. Hough [10] reports a similar observation. The sugar (300 mg.) was dissolved in water (6 ml.), and a solution of potassium borohydride (100 mg.) in water (2 ml.) was added. After 45 min. excess of borohydride was destroyed by the addition of dilute acetic acid, and the

solution was taken to dryness. The residue was heated at 100° for 30 min. with acetic anhydride (12 ml.) and anhydrous sodium acetate (150 mg.). The solution was poured into ice-water; the precipitate after recrystallisation from ethanol–light petroleum (b. p. 80—100°) and from ethanol had m. p. 112·5—115° and $[\alpha]_D^{18}$ −24° (*c* 2·0 in $CHCl_3$) {Whistler and Smith [3] report m. p. 113·5—114° and $[\alpha]_D^{25}$ −21° (in $CHCl_3$) for mannotri-itol dodeca-acetate}.

Fraction 4. The syrup (1·84 g.; eluted with water containing 10·5—11·5% of ethanol) contained three components, mannotriose, and trisaccharides C and D, having $R_{mannose}$ 0·22, 0·29, and 0·15 respectively. Chromatographically pure samples of trisaccharides C and D were obtained after fractionation on cellulose with solvent C. Trisaccharide C crystallised from ethanol water and had m. p. 224—225° and $[\alpha]_D^{18}$ +43° (5 min.) ⟶ +40° (1 hr., const.) (*c* 2·1 in H_2O) (Found: C, 42·9; H, 6·4. $C_{18}H_{32}O_{16}$ requires C, 42·8; H, 6·4%). Partial acid hydrolysis of the trisaccharide yielded mannose, mannobiose, and disaccharide A; partial acid hydrolysis of the derived glycitol (borohydride reduction) gave mannose and disaccharide A. When oxidised with periodate [11] the trisaccharide consumed 5·0 mol. after 8 hr. and 6·0 mol. after 24 hr.; *O*-β-D-mannopyranosyl-(1 ⟶ 4)-*O*-β-D-mannopyranosyl)-(1 ⟶ 4)-D-mannopyranose trihydrate consumed 5·0 ml. after 8 hr. and 5·95 mol. after 24 hr. Trisaccharide C (100 mg.) was dissolved in water (4 ml.) and a solution of potassium borohydride (40 mg.) in water (1 ml.) was added. The reaction mixture was kept at room temperature for 1 hr., excess of borohydride was destroyed by the addition of acetic acid, and inorganic salts were removed by passage through columns of Amberlite resins IR-100(H) and IR-4B(OH). The reduction product was methylated by successive additions of methyl sulphate and sodium hydroxide. The methylated glycitol (*ca.* 120 mg.), isolated from the mixture by chloroform extraction, was hydrolysed by 0·5N-sulphuric acid (10 ml.) for 3 hr. at 100°, and the hydrolysate was neutralised with barium carbonate, deionised, and concentrated. Separation of the reducing sugars in the hydrolysate on filter sheets with solvent C gave fractions *a* (42 mg.) and *b* (31 mg.). Fraction *a* was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose by conversion into the aniline derivative, m. p. and mixed m. p. 142—144°. Fraction *b* was identified as 2 : 3 : 6-tri-*O*-methyl-D-mannose by conversion into the di-*p*-nitrobenzoate, m. p. and mixed m. p. 183—185°.

Trisaccharide D had $[\alpha]_D^{17}$ −7·0° (*c* 4·1 in H_2O) and gave on hydrolysis mannose and glucose in the approximate ratio of 2 : 1. Partial acid hydrolysis of the trisaccharide yielded mannose, glucose, mannobiose, and disaccharide B; partial acid hydrolysis of the derived glycitol gave mannose and mannobiose.

Fraction 5. The sugar (2·1 g.; eluted with water containing 11·5—13·0% of ethanol) crystallised from ethanol–water, and after several recrystallisations had $R_{mannose}$ 0·11 in solvent A, m. p. 231·5—232°, and $[\alpha]_D^{20}$ −31·6° (5 min.) ⟶ −28·7° (50 min., const.) (*c* 0·9 in H_2O). The sugar gave only mannose on hydrolysis and after partial acid hydrolysis chromatography showed the homologous series, mannose, mannobiose, mannotriose, and unchanged tetrasaccharide.

Fraction 6. The syrup (0·9 g.; eluted with water containing 13·0—14·5% of ethanol) contained mannotetraose together with samll quantities of a faster-moving component ($R_{mannose}$ 0·17). The minor component, separated on filter sheets with solvent A, gave only mannose on hydrolysis, and on partial acid hydrolysis mannose, mannobiose, mannotriose, disaccharide A, and trisaccharide C could be detected by chromatography.

Fraction 7. The sugar (0·9 g.; eluted with water containing 15—16% of ethanol) crystallised from ethanol–water and had $[\alpha]_D^{20}$ −30·2° (*c* 2·1 in H_2O); no m. p. (>280°) could be recorded. On partial acid hydrolysis, the homologous series of oligosaccharides, mannose, mannobiose, mannotriose, mannotetraose, and unchanged pentasaccharide were detected chromatographically.

Fraction 8. The syrup (0·8 g.; eluted with water containing 17—18% of ethanol) contained a complex mixture of oligosaccharides including a component having $R_{mannose}$ 0·08. A small quantity of this sugar was separated on filter sheets with solvent A and yielded on partial acid hydrolysis mannose, mannobiose, mannotriose, disaccharide A, and trisaccharide C.

Acetolysis of Mannan B and Fractionation of Derived Oligosaccharides.—Mannan B (13 g.) was added slowly to a mixture of acetic anhydride (80 ml.), glacial acetic acid (80 ml.), and concentrated sulphuric acid (8 ml.) at 0°. After 96 hr. at room temperature, the mixture was poured into ice-water (750 ml.), and sodium hydrogen carbonate was added slowly (to pH 3—4). The precipitated sugar acetates were filtered off and dissolved in chloroform, and the solution was combined with a chloroform extract (3 × 500 ml.) of the aqueous filtrate. The chloroform

solution was washed with aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated to give a mixture of sugar acetates (14·6 g.). A portion of the sugar acetates (10·1 g.) was dissolved in dry methanol (150 ml.), and a solution of barium methoxide (1·35 g.) in methanol (15 ml.) was added. The mixture was shaken for 1 hr. and set aside at 0°. The mixture was worked up as described previously for mannan A and yielded a mixture of sugars (4·8 g.). The mixture of sugars dissolved in water (150 ml.) was added to a column of charcoal–Celite (300 g.; 1 : 1). Elution with water and ethanol–water (2 : 98) gave a mixture of monosaccharides (mannose, together with traces of xylose, glucose, and galactose; 0·42 g.) which was not examined further. Oligosaccharides were eluted with ethanol–water containing increasing proportions of ethanol. Elution with water containing 4—6% of ethanol gave 4-*O*-β-D-mannopyranosyl-D-mannopyranose (0·92 g.), m. p. and mixed m. p. 203—206°, which gave an *X*-ray powder photograph identical with that of an authentic sample. Elution with water containing 7—8% of ethanol yielded a syrup (84 mg.) shown by chromatography to contain three disaccharides (mannobiose, and disaccharides A and B) having $R_{mannose}$ 0·52, 0·62, and 0·38 in solvent A. Elution with water containing 9% of ethanol gave mannotriose trihydrate (600 mg.), which, after recrystallisation from ethanol–water, had m. p. and mixed m. p. 134·5—135·5°, and gave an *X*-ray powder identical with that of an authentic sample. Elution with water containing 10—11% of ethanol gave a mixture (166 mg.) of mannotriose, and trisaccharides C and D having $R_{mannose}$ 0·22, 0·29, and 0·15. Elution with water containing 12% of ethanol afforded mannotetraose, which, after recrystallisation from ethanol–water, had m. p. and mixed m. p. 231·5—232° and gave an *X*-ray powder photograph identical with that of an authentic sample.

Tests for Acid Reversion during Acetolysis.—Samples of mannose, mannobiose, and mannotriose (150—200 mg.) were each dissolved in 2 ml. of acetolysis mixture [acetic anhydride, acetic acid, and concentrated sulphuric acid, 10 : 10 : 1 (v/v)] at 0°. The solutions were kept at room temperature for 48 hr., filtered, and poured into ice-water, and sodium hydrogen carbonate solution was added. The sugar acetates, isolated by chloroform extraction, were deacetylated with barium methoxide in methanol at 0° for 18 hr., and the mixture was poured into water. The resulting solution was deionised by passage through columns of Amberlite resins IR-120(H) and IR-4B(OH) and by electrodialysis with ion-exchange membranes,[12] and concentrated. Chromatographic examination of the products in solvent A showed only starting materials and hydrolysis products.

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47. *Gum Ghatti (Indian Gum). Part II.* The Hydrolysis Products obtained from the Methylated Degraded Gum and the Methylated Gum.*

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Hydrolysis of methylated degraded gum ghatti yields 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, 2 : 3-, and 2 : 4-di-*O*-methyl-D-galactose, 3 : 4 : 6-tri-*O*-methyl-D-mannose, and 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, together with traces of other sugars. Hydrolysis of methylated gum ghatti affords 2 : 3 : 5-tri-, 2 : 3-, 2 : 4-, 2 : 5-, and 3 : 5-di-*O*-methyl-L-arabinose, 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, 2 : 4-di-, and 2-mono-*O*-methyl-D-galactose, 4-*O*-methyl-D-mannose, 2 : 3 : 4-tri-*O*-methyl-L-rhamnose, 2 : 3 : 4-tri-, and 2 : 3-di-*O*-methyl-D-glucuronic acid, together with traces of other sugars. Partial structures for the gum are discussed in the light of these and previous results.

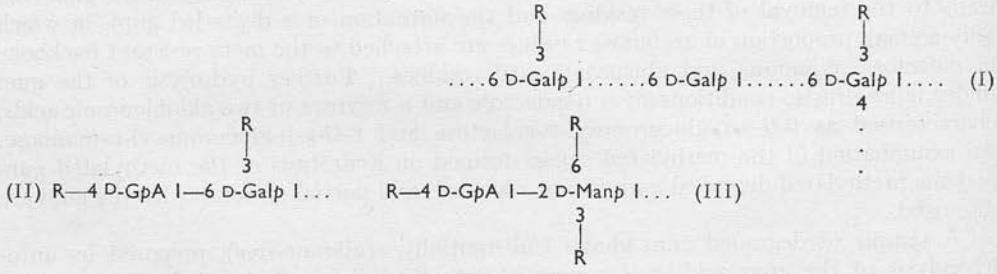
It was shown in Part I that gum ghatti (Indian gum) from *Anogeissus latifolia*, Wall, is composed of the following sugar residues, L-arabinose (5 parts), D-galactose (3 parts), D-mannose (1 part), and D-glucuronic acid (1 part), together with the small amounts of xylose and a 6-deoxyhexose (probably rhamnose). Most of the arabinose residues are located in the outer parts of the molecular structure since autohydrolysis of the gum acid leads to the removal of these residues and the formation of a degraded gum, in which only a small proportion of arabinose residues are attached to the more resistant backbone of galactose, mannose, and glucuronic acid residues. Further hydrolysis of the gum under more drastic conditions gives D-galactose and a mixture of two aldobiouronic acids, characterised as 6-*O*-β-D-glucuronosyl-D-galactose and 2-*O*-β-D-glucuronosyl-D-mannose. An examination of the methylated sugars formed on hydrolysis of the methylated gum and the methylated degraded gum is now reported, and partial structures for the gum are discussed.

A sample of degraded gum ghatti (substantially arabinose-free), prepared by autohydrolysis of the gum acid, was converted into its fully methylated derivative. The methylated polysaccharide was hydrolysed, and the hydrolysate (containing acidic components as barium salts) was separated into an ether-soluble fraction and an ether-insoluble residue. The neutral methylated sugars present in the ether-soluble fraction were fractionated chromatographically on cellulose,[1] giving 2 : 3 : 4 : 6-tetra-*O*-methyl-, 2 : 3 : 4-tri-*O*-methyl-, 2 : 3- and 2 : 4-di-*O*-methyl-D-galactose, all of which were characterised as crystalline derivatives. Although no methyl ethers of D-mannose could be isolated in pure form and identified as crystalline derivatives, the presence of 3 : 4 : 6-tri-*O*-methylmannose was established by chromatography, paper ionophoresis, the detection of 2 : 3 : 5-tri-*O*-methylarabinose when a fraction containing the sugar was oxidised with periodate,[2] and the observation that a fraction containing the sugar in admixture with tetra- and 2 : 3 : 4-tri-*O*-methylgalactose was converted into the corresponding mixture of aldonamides, which gave a positive Weerman test. This sugar was the most important mannose derivative present, although traces of a di-*O*-methyl- (probably 3 : 4-) and 4-*O*-methyl-mannose were also observed. In addition, traces of 2 : 3 : 5-tri-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methyl-xylose and rhamnose were detected chromatographically, but in insufficient quantity to be of structural significance. The ether-insoluble residue afforded further quantities of 2 : 3 : 4-tri- and di-*O*-methylgalactose, which were separated from the barium salts on filter sheets. The acidic fraction, consisting mainly of methylated aldobiouronic acids, was submitted to further acid hydrolysis under vigorous conditions, and again the acidic were separated from the neutral sugars.

* The paper by Aspinall, Hirst, and Wickstrøm (*J.*, 1955, 1160) is to be regarded as Part I.

The neutral fraction contained 2 : 3 : 4-tri-*O*-methyl-D-galactose, identified as the aniline derivative, and 3 : 4 : 6-tri-*O*-methylmannose and 2 : 3-di-*O*-methylgalactose were identified chromatographically. The main component of the acidic fraction was identified chromatographically as 2 : 3 : 4-tri-*O*-methylglucuronic acid, and, after reduction of the methyl ester methyl glycoside and hydrolysis, as 2 : 3 : 4-tri-*O*-methylglucose. The minor component of the acidic fraction was converted into the methyl ester methyl glycoside, reduced with lithium aluminium hydride, and hydrolysed to give 2 : 3-di-*O*-methylglucose together with small amounts of tri-*O*-methylglucose and tri- and di-*O*-methylgalactose. Some of the glucuronic acid residues in the degraded gum, therefore, were present in non-terminal positions linked through $C_{(1)}$ and $C_{(4)}$.

It is clear from these results that the gum contains a backbone of 1 : 6-linked D-galactopyranose units (I; R = H). Although both 2 : 3- and 2 : 4-di-*O*-methyl-D-galactose were characterised, evidence from ionophoresis and from chromatographic examination of the periodate oxidation products of the di-*O*-methylgalactose fractions showed the 2 : 3-dimethyl ether to be the main component of the mixture. (When mixtures of these two sugars are present, the 2 : 4-dimethyl ether is more readily characterised as the relatively insoluble aniline derivative.) It follows that the main branching point in the degraded gum is through position 4 of galactose, although some small proportion of branching may also occur through position 3. Since the main products of hydrolysis of the methylated aldobiouronic acids were 2 : 3 : 4-tri-*O*-methylglucuronic acid, 2 : 3 : 4-tri-*O*-methylgalactose, and 3 : 4 : 6-tri-*O*-methylmannose, the aldobiouronic acid groups are



present as terminal groups (II and III; R = H). These groups are, therefore, attached as side-chains probably through position 4 of galactose residues in the backbone. At present, there is no indication whether these units are attached directly to the backbone or whether 1 : 6-linked galactose residues are interposed. The structural significance of the non-reducing D-galactopyranose end groups in the degraded gum is not yet clear. Although these end groups may terminate another type of side-chain, it is also possible that they are present at the non-reducing end of the backbone and arise from scission of the main chain during the autohydrolysis.

Fully methylated gum ghatti was hydrolysed, and the hydrolysate (containing acidic components as barium salts) was separated into an ether-soluble fraction (A) and an ether-insoluble residue (B). The residue (B) was then separated by chromatography on cellulose to give neutral sugars (C), a pure sample of 2-*O*-methyl-D-galactose, and an acidic fraction. The combined neutral sugars (A) + (C) were chromatographed on cellulose, giving pure samples of most of the major components and mixtures containing the minor components, which were refractionated. The following methylated sugars were characterised as crystalline derivatives: 2 : 3 : 5-tri-, 2 : 3-, 2 : 4-, 2 : 5-, and 3 : 5-di-*O*-methyl-L-arabinose, 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, 2 : 4-di-, and 2-mono-*O*-methyl-D-galactose, 4-*O*-methyl-D-mannose, and 2 : 3 : 4-tri-*O*-methyl-L-rhamnose. Traces of some other sugars were detected chromatographically, but these were present in insufficient amount to be of structural significance. The acidic fraction, which was contaminated by a small amount of 2-*O*-methylgalactose, was converted into the corresponding mixture of

methyl ester methyl glycosides, which was reduced with lithium aluminium hydride and hydrolysed to give a mixture of neutral sugars. The methylated sugars were fractionated on cellulose, and the following sugars were identified as crystalline derivatives: 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-D-glucose, 2 : 4-di- and 2-mono-*O*-methyl-D-galactose, and 4-*O*-methyl-D-mannose. The isolation of 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-D-glucose indicates the presence in the methylated gum of residues of 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-D-glucuronic acid. 2 : 4-Di-*O*-methyl-D-galactose and 4-*O*-methyl-D-mannose are the main neutral fragments arising from the aldobiouronic acid groupings. It is probable that the 2-*O*-methyl-D-galactose arose entirely from the contaminating neutral sugar and not from hydrolysis of a partially methylated aldobiouronic acid. In addition, a trace of 2 : 3 : 4-tri-*O*-methylgalactose was detected chromatographically

In view of the several stages involved in the separation and identification of the many methylated sugars formed on hydrolysis of the methylated gum, it is not possible to give more than an approximate estimate of the proportions of some of the constituent sugars arising from the neutral part of the gum. Since even more operations were involved in the identification of the acidic residues and of the neutral sugar residues attached thereto, and since the hydrolyses of acidic polysaccharides are usually accompanied by some decomposition, it is again only possible to estimate their relative proportions approximately. The significance of these results, taken together with previous results, may be assessed most conveniently by considering, in turn, the L-arabinose residues removed during the autohydrolysis, the backbone of 1 : 6-linked D-galactopyranose residues, and the aldobiouronic acid units. The approximate composition of the gum determined in Part I and expressed as parts per equivalent weight of gum acid provides a useful working model.

The quantity of 2 : 3 : 5-tri-*O*-methyl-L-arabinose isolated from the methylated gum accounts for approximately four of the five parts of L-arabinose present per equivalent of gum. Although relatively small amounts of 2 : 3 : 4-tri-*O*-methyl-L-rhamnose, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, and 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid were also present as units of the methylated gum, terminal L-arabofuranose residues account for most of the non-reducing end groups in the gum. The fifth part of L-arabinose is accounted for by approximately equal amounts of 2 : 3-, 2 : 4-, 2 : 5-, and 3 : 5-di-*O*-methyl-L-arabinose. Three of these four sugars represent units not susceptible to attack by periodate. Since it was shown in Part I that about 20% of the arabinose residues in the gum are not attacked by periodate, it follows that most, if not all, of these sugars are of structural significance and do not arise from incomplete methylation of the gum or from demethylation during hydrolysis. These non-terminal arabinose residues must also occur in the outer parts of the molecule since they are removed as free arabinose during the autohydrolysis of the gum acid. It is clear, therefore, that the majority of L-arabinose residues in the gum occur in the furanose form as single-unit side-chains attached to the more resistant part of the structure. In a few cases, however, non-terminal L-arabinose residues must be interposed between the end groups and the other sugar residues. The small proportion of L-arabopyranose residues in the gum, as shown by the isolation of 2 : 4-di-*O*-methyl-L-arabinose, is of particular interest as until recently [3] L-arabinose had been found in combination only in the furanose form.

The D-galactose residues present in the gum occur in three main types of combination as shown by the isolation of 2 : 3 : 4-tri- (<1 part), 2 : 4-di- (>1 part), and 2-mono- (>1 part) *O*-methyl-D-galactose from the methylated gum. Since the corresponding residues in the methylated degraded gum afford 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-D-galactose, it follows that the preferred mode of attachment of arabinose is to position 3 of galactose, although it is possible that some arabinose residues may also be linked to position 4. It is probable, however, that most of the 2-*O*-methyl-D-galactose represents a double branching point, to arabinose through position 3, and to aldobiuronic acid through position 4. These results are in reasonable agreement with the results of periodate

oxidation of the gum (Part I) where it was shown that about a third of the galactose residues in the gum were attacked by periodate. There is no evidence at present as to the rôle of the very small proportion of D-galactopyranose end groups.

The sugar residues present in the aldobiouronic acid groupings in the methylated gum are those of 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, 2 : 3-di-*O*-methyl-D-glucuronic acid (main acid component), 2 : 4-di-*O*-methyl-D-galactose, and 4-*O*-methyl-D-mannose, whereas the corresponding residues in the methylated degraded gum are those of 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid (main acid component), 2 : 3-di-*O*-methyl-D-glucuronic acid, 2 : 3 : 4-tri-*O*-methyl-D-galactose, and 3 : 4 : 6-tri-*O*-methyl-D-mannose. It follows that arabinose residues are attached to these sugars through position 3 of galactose, positions 3 and 6 of mannose, and through position 4 of some glucuronic acid residues. Apart from chromatographic and ionophoretic evidence for traces of 3 : 4 : 6-tri-*O*-methylmannose, the only derivative of D-mannose found in the hydrolysate from the methylated gum was the 4-methyl ether, indicating one main mode of linkage of mannose residues in the gum. The evidence adduced in Part I indicated, on the one hand, the presence of one part of mannose and one part of glucuronic acid per equivalent of gum, and, on the other hand, the linking of glucuronic acid to both galactose and mannose, and suggested that some mannose residues in the gum may be linked to neutral sugar residues only. Experiments to provide further evidence on this point are in progress.

In Part I it was shown that hydrolysis of gum ghatti affords small amounts of xylose and a 6-deoxyhexose (probably rhamnose) in addition to the main constituent sugars. The isolation of 2 : 3 : 4-tri-*O*-methyl-L-rhamnose from the methylated gum confirms the presence of L-rhamnose residues in the gum. No other methyl ethers of rhamnose were detected. Since this sugar has persisted throughout the various operations it seems probable that it is an integral part of the gum structure and does not arise from a contaminating polysaccharide. On the other hand, only traces of xylose derivatives (as the 2 : 3 : 4-trimethyl ether) could be detected on hydrolysis of the methylated gum. It is unlikely, therefore, that xylose is a constituent of the gum itself.

Our present knowledge of the detailed molecular structure of gum ghatti may be summarised in terms of the partial structures (I, II, and III), with the substituent groups R representing mainly single L-arabofuranose residues, but in a few cases more complex arabinose-containing side-chains terminated again by L-arabofuranose residues. Experiments to determine the mode of attachment of the aldobiouronic acid side-chains (II and III) to the backbone of galactose residues (I) will be reported later. It is already clear that gum ghatti resembles several other plant gums, notably damson, cherry, and egg-plum gums,[4-6] in containing a high proportion of L-arabofuranose residues in the outer parts of the molecular structure. It differs, however, from these gums in containing galactose residues mutually linked mainly through $C_{(1)}$ and $C_{(6)}$, and not through $C_{(3)}$ also. Gum ghatti differs also in this respect from gum arabic, which is now known to contain a backbone of 1 : 3-linked D-galactopyranose units to which are attached side-chains of 1 : 6-linked galactose units.[7]

Experimental

Paper chromatography was carried out on Whatman No. 1 filter paper using the following solvent systems (v/v): (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer); (B) ethyl acetate–acetic acid–water (3 : 1 : 3, upper layer); (C) butan-1-ol–acetic acid–water (4 : 1 : 5, upper layer); (D) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (E) benzene–ethanol–water (169 : 47 : 15, upper layer); (F) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (G) butan-2-one, half saturated with water containing 1% of ammonia. Unless otherwise stated, chromatography of methylated sugars was carried out in solvent D and R_G values refer to rate of movement relative to 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose in that solvent. Demethylations of methylated sugars were carried out by the procedure of Hough, Jones, and Wadman.[8] Paper ionophoresis was carried out in borate buffer at pH 10 at a

potential of 500 v. Aniline derivatives of methylated sugars were prepared by refluxing the sugar in ethanolic aniline for 30 min.; further heating resulting in darkening of the solution. Optical rotations were observed at 18° ± 2°.

Samples of the gum acid and the degraded gum acid were prepared as described in Part I. During the preparation of the degraded gum acid the autohydrolysis of the gum acid caused the release of arabinose and only traces of xylose, rhamnose, and galactose. The various samples of degraded gum acid had slightly different optical rotations, $[\alpha]_D$ (as barium salt) varying from +2° to +9°. These samples were combined for subsequent experiments since the chromatographic patterns, after hydrolysis with N-sulphuric acid for 6 hr. at 100°, were similar in each case showing galactose in quantity, two aldobiouronic acids, small amounts of arabinose and mannose, and a trace of xylose.

Preparation and Hydrolysis of Methylated Degraded Gum.—The degraded gum (15 g.) was methylated extensively with methyl sulphate and sodium hydroxide following the procedure of Brown, Hirst, and Jones.[9] The product isolated as methylated degraded gum acid (8·2 g.; OMe, 36·9%) was further methylated with methyl iodide and silver oxide, giving methylated degraded gum (6·7 g.). Fractional precipitation of the methylated polysaccharide from chloroform by light petroleum gave fraction (*a*) (3·7 g.; OMe, 44·4%), which had $[\alpha]_D$ −28° (*c* 1·0 in $CHCl_3$), and fraction (*b*) (3·0 g.; OMe, 45·1%), which had $[\alpha]_D$ −11·5° (*c* 1·0 in $CHCl_3$). Chromatographic examination of the hydrolysates of the two fractions in solvent D showed similar complex mixtures of sugars.

Methylated degraded gum (fraction *a*; 3·2 g.) was suspended in N-hydrochloric acid (500 ml.) at 35° for 7 days, and the resulting solution was heated at 100° for 12·5 hr. (constant rotation), cooled, neutralised with silver carbonate, and filtered, and the filtrate was treated with hydrogen sulphide to remove silver ions, filtered, and concentrated. The resulting syrup was dissolved in water, and the solution was neutralised with barium carbonate, filtered, and concentrated to a dark syrup (3·0 g.). The dry syrup was repeatedly extracted with dry ether, to give an ether-soluble fraction (1·92 g.) and an ether-insoluble residue.

Examination of the Ether-soluble Fraction.—The syrup was separated on cellulose (60 × 3·4 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later 1 : 1) saturated with water, and butan-1-ol partly saturated with water, as eluants, to give eight fractions, and a further fraction was obtained by elution of the cellulose with water.

Fraction 1. The syrup (23 mg.) contained a mixture of sugars (R_G 0·97—0·93) and a trace of tri-*O*-methylgalactose (R_G 0·72). Chromatographic examination in solvent E showed 2 : 3 : 4-tri-*O*-methylxylose and two sugars having similar mobilities and staining properties with aniline oxalate to 2 : 3 : 4-tri-*O*-methylrhamnose and 2 : 3 : 5-tri-*O*-methylarabinose. Demethylation gave galactose and traces of xylose and arabinose. The remainder of the syrup was hydrolysed with N-hydrochloric acid at 100 for 6 hr., and chromatography showed much di-*O*-methylgalactose in the hydrolysate. The origin of the latter sugar is obscure.

Fraction 2. The syrup (97 mg.) had $[\alpha]_D$ +100° (*c* 0·34) and R_G 0·89. The sugar was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 189—190°.

Fraction 3. Chromatography of the syrup (92 mg.) showed three components corresponding to 2 : 3 : 4 : 6-tetra-*O*-methylgalactose, 3 : 4 : 6-tri-*O*-methylmannose, and 2 : 3 : 4-tri-*O*-methylgalactose, R_G 0·89, 0·82, and 0·72. Demethylation gave galactose and mannose. Paper ionophoresis showed a component having the same mobility as 3 : 4 : 6-tri-*O*-methyl-D-mannose. Chromatographic examination of the products of periodate oxidation [2] showed 2 : 3 : 5-tri-*O*-methylarabinose (R_G 0·95) in addition to unchanged starting material. The syrup (50 mg.) was converted into the corresponding mixture of aldonamides, which, after treatment with sodium hypochlorite and addition of semicarbazide, afforded hydrazodicarbonamide, m. p. 268° and mixed m. p. (with sample of m. p. 263—264°) 264°.

Fraction 4. The chromatographically pure syrup (0·904 g.) had $[\alpha]_D$ +118° (*c* 0·42) and R_G 0·72 (Found: OMe, 41·7. Calc. for $C_9H_{18}O_6$: OMe, 41·8%). Demethylation gave only galactose. The sugar was identified as 2 : 3 : 4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. 165—167° and mixed m. p. (with sample of m. p. 159—161°) 159—162°. The sugar subsequently crystallised from acetone–ether as the monohydrate, m. p. 71° and mixed m. p. (with sample m. p. 73—76°) 72—73°.

Fraction 5. The syrup (112 mg.) contained two components, R_G 0·72 (2 : 3 : 4-tri-*O*-methylgalactose) and 0·60. The second component showed similar chromatographic and iono-

phoretic behaviour to 3 : 4-di-*O*-methyl-D-mannose. The mixture gave galactose and mannose on demethylation. Attempts to separate the two components by chromatography on Amberlite resin IRA-400 (borate form) were unsuccessful.

Fraction 6. The syrup (203 mg.) contained two components, having R_G 0·49 and 0·75 respectively, the coloration of the former with aniline oxalate suggesting 2 : 3-di-*O*-methylgalactose. Demethylation gave only galactose. Separation of the syrup (190 mg.) on filter sheets using solvent D gave fractions 6*a* (70 mg.) and 6*b* (54 mg.). Paper ionophoresis of fraction 6*a* showed 2 : 3-di-*O*-methylgalactose and a trace of the 2 : 4-dimethyl ether. Chromatographic examination of the products of periodate oxidation [2] confirmed the presence of 2 : 3-di-*O*-methylgalactose. An authentic sample of 2 : 3-di-*O*-methyl-D-galactose when oxidised with periodate showed three oxidation products with R_F 0·66 (grey), 0·78 (brown), and 0·87 (grey) respectively, whereas 2 : 4-di-*O*-methyl-D-galactose showed only unchanged sugar (R_F 0·37). The sugar had $[\alpha]_D$ +69° ⟶ +80° (*c* 0·37) and the identity of the main component was proved by conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. 125—128° and mixed m. p. 124—127°. Fraction 6*b* was hydrolysed with N-hydrochloric acid for 6 hr. at 100° and chromatographic examination showed only 2 : 3 : 4-tri-*O*-methylgalactose; it is probable that this fraction contains a polymer of 2 : 3 : 4-tri-*O*-methyl-D-galactose arising from incomplete hydrolysis of the methylated polysaccharide.

Fraction 7. The syrup (91 mg.) contained at least two components (having R_G 0·48 and 0·75 respectively). Separation of the mixture (88 mg.) on filter sheets using solvent D gave fractions 7*a* (53 mg.) and 7*b* (17 mg.). Paper ionophoresis and chromatographic examination of the products of periodate oxidation [2] indicated the presence in fraction 7*a* of 2 : 3- and 2 : 4-di-*O*-methylgalactose. The presence in the mixture of 2 : 4-di-*O*-methyl-D-galactose was shown by conversion into the aniline derivative, m. p. and mixed m. p. 202—204°. Fraction 7*b* contained the same substance (R_G 0·75) as fraction 6*b* and small amounts of di-*O*-methylgalactose. On hydrolysis the main component gave 2 : 3 : 4-tri-*O*-methylgalactose.

Fraction 8. The syrup (31 mg.) contained a di-*O*-methylgalactose (R_G 0·45) and small amounts of 4-*O*-methylmannose and an unidentified sugar (R_G 0·65).

Fraction 9. The syrup (48 mg.) contained a complex mixture of acidic and neutral sugars and was not examined further.

Examination of the Ether-insoluble Fraction.—The ether-insoluble residue was treated with Amberlite resin IR-120(H) to remove barium ions, and concentration gave a syrup (0·714 g.) (equiv. wt., 597). Chromatography showed neutral sugars in addition to acidic substances. The syrup (0·63 g.) was dissolved in water and neutralised with barium carbonate, and the resulting mixture was separated on filter sheets using solvent D, to give barium salts (A) (365 mg.), and fractions B (i) (190 mg.) and B (ii) (92 mg.), both contaminated with acidic substances. Chromatography showed fraction B (i) to contain mainly 2 : 3 : 4-tri-*O*-methylgalactose (R_G 0·72), and fraction B (ii) to contain di-*O*-methylgalactose (ionophoresis and chromatography of the periodate oxidation products [2] indicating the 2 : 3-isomer to be the main component). A sample (25 mg.) of barium salts (A) was treated with Amberlite resin IR-120(H) to give a mixture of acids (equiv. wt., 370, indicating mainly aldobiouronic acid) which on hydrolysis yielded acidic and neutral sugars.

Barium salts (A) (330 mg.) were converted into the corresponding acids (307 mg.) which were hydrolysed with N-hydrochloric acid at 100° for 7 hr. After neutralisation with silver carbonate, separation on filter sheets in solvent D afforded neutral sugars [fractions C (i)—C (iv)] and silver salts (D). The silver salts (D) were converted into acids (143 mg.) which were separated on filter sheets by solvent C into fractions D (i) (48 mg.) and (D) (ii) (36 mg.).

Fraction C (i) (9 mg.) contained 3 : 4 : 6-tri-*O*-methylmannose (R_G 0·82). Fraction C (ii) (35 mg.) contained 2 : 3 : 4-tri-*O*-methyl-D-galactose (R_G 0·72), identified as the aniline derivative, m. p. 159—162° and mixed m. p. 156—159°. Fraction C (iii) (26 mg.) contained 2 : 3 : 4-tri-*O*-methylgalactose (R_G 0·72) and a trace of a second component (R_G 0·60). Fraction C (iv) (28 mg.) contained di-*O*-methylgalactose (R_G 0·49), shown by ionophoresis and chromatography of the periodate oxidation products [2] to be mainly the 2 : 3-dimethyl ether. Fraction (i) was chromatographically pure 2 : 3 : 4-tri-*O*-methylglucuronic acid. Attempts to characterise the sugar by conversion into the crystalline amide of methyl 2 : 3 : 4-tri-*O*-methyl-α-D-glucuronoside failed. The syrupy product was treated with methanolic hydrogen chloride, and the resulting ester was reduced with lithium aluminium hydride and hydrolysed. Chromatography showed only 2 : 3 : 4-tri-*O*-methylglucose (R_G 0·85). Fraction D (ii), which contained at least

three components, was converted into the methyl ester methyl glycosides, reduced with lithium aluminium hydride, and hydrolysed. Chromatography showed 2 : 3-di-*O*-methylglucose, small amounts of tri- and di-*O*-methylgalactose, and a trace of 2 : 3 : 4-tri-*O*-methylglucose.

Preparation and Hydrolysis of Methylated Gum.—The gum acid (25 g.) was methylated extensively with methyl sulphate and sodium hydroxide by the procedure of Brown, Hirst, and Jones.[9] The product isolated as the methylated gum acid (18 g.; OMe, 35·0%; ash, 5·8%) was further methylated with methyl iodide and silver oxide (three treatments) to give methylated gum (7·3 g.), $[\alpha]_D$ −72° (*c* 1·0 in $CHCl_3$) (Found: OMe, 42·8%).

The methylated gum (7·3 g.) was refluxed with methanolic 2% hydrogen chloride (500 ml.) for 12 hr. (constant rotation). Methanol was removed under reduced pressure and the product was heated with 0·55N-hydrochloric acid (600 ml.) on the boiling-water bath for 12 hr. (constant rotation). The cooled solution was neutralised with silver carbonate, then filtered, and hydrogen sulphide was passed through the filtrate to precipitate silver ions, and the filtrate was concentrated. Sugars were extracted from the residue with methanol, and the resulting syrup was dissolved in water, neutralised with barium carbonate, filtered, and concentrated to a syrup (7·3 g.).

The mixture of methylated sugars (7·3 g.) was repeatedly extracted with dry ether to give ether-soluble sugars (A) (4·76 g.) and an ether-insoluble residue (B) (2·10 g.). The ether-insoluble sugars (B) were separated into neutral and acid fractions by chromatography on cellulose (50 × 2·5 cm.) with butan-1-ol, 80% saturated with water, as eluant, four fractions being isolated. Fraction (C) (0·80 g.) contained a mixture of neutral sugars. Fraction (D) (0·224 g.) contained chromatographically pure 2-*O*-methyl-D-galactose, m. p. 157—158° (from acetone–water), $[\alpha]_D$ +55° (5 min.) ⟶ +89° (120 min., const.) (*c* 1·91 in H_2O). Fraction (E) (0·14 g.) contained a mixture of 2-*O*-methylgalactose and acidic sugars. Fraction (F) (0·398 g.) contained acidic components and a trace of 2-*O*-methylgalactose.

Examination of Neutral Methylated Sugars.—The ether-soluble sugars (A) and fraction (C) were combined and separated on cellulose (76 × 3·5 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later, 1 : 1) saturated with water, and butan-1-ol partly saturated with water as eluants, to give eighteen fractions. A further fraction (19) (64 mg.) was obtained by elution of the cellulose with water.

Fraction 1. The syrup (119 mg.) had $[\alpha]_D$ +15° (*c* 0·39), and chromatography showed a main component having R_G 1·03 (cf. 2 : 3 : 4-tri-*O*-methyl-L-rhamnose) and traces of other sugars. Chromatography in solvent E showed a second component travelling faster. Hydrolysis of a sample with N-sulphuric acid, followed by neutralisation with barium carbonate and chromatography showed tri-*O*-methylrhamnose, 2 : 3 : 5-tri-*O*-methylarabinose, and the barium salt of an acid (at the starting line of the paper). Treatment of a second sample with cold barium hydroxide, followed by neutralisation with carbon dioxide and chromatography, gave a similar result. The remaining syrup (*ca.* 80 mg.) was treated with cold 5% barium hydroxide solution for 30 min., and the solution was neutralised with carbon dioxide, filtered and concentrated. The product was separated on a filter sheet with solvent D, to give fractions 1*a* and 1*b*. Fraction 1*a* contained 2 : 3 : 4-tri-*O*-methylrhamnose (R_G 1·03) and a trace of 2 : 3 : 5-tri-*O*-methylarabinose (R_G 0·97). The main component was identified by conversion into 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-L-rhamnosylamine, m. p. and mixed m. p. 98—100°. Fraction 1*b* (barium salt) was deionised with Amberlite resin IR-120(H), and chromatography in solvent C showed 2 : 3 : 4-tri-*O*-methylglucuronic acid. Conversion of the acid into the methyl ester methyl glycoside with dry methanolic hydrogen chloride, followed by reduction with lithium aluminium hydride in methylal, hydrolysis with N-hydrochloric acid, and chromatography in solvent D, showed only 2 : 3 : 4-tri-*O*-methylglucose. It is concluded that tri-*O*-methylglucuronic acid was present in fraction 1 as an ester.

Fraction 2. Chromatography of the syrup (150 mg.) showed 2 : 3 : 5-tri-*O*-methylarabinose and a trace of 2 : 3 : 4-tri-*O*-methylrhamnose. The optical rotation, $[\alpha]_D$ −41° (*c* 0·59), indicated almost pure 2 : 3 : 5-tri-*O*-methyl-L-arabinose (cf. 2 : 3 : 5-tri-*O*-methyl-L-arabinose,[10] $[\alpha]_D$ −39·5°).

Fraction 3. The syrup (1·835 g.), which had $[\alpha]_D$ −42·5° (*c* 0·68), was almost pure 2 : 3 : 5-tri-*O*-methyl-L-arabinose with a trace of 2 : 3 : 4 : 6-tetra-*O*-methylgalactose. Demethylation gave arabinose and a trace of galactose. The identity of the main component was confirmed by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. 132—133° and mixed m. p. (with sample of m. p. 129—130°) 129°.

Fraction 4. Chromatography of the syrup (34 mg.), which had $[\alpha]_D$ +66° (*c* 0·48), showed a single component, R_G 0·97. Re-examination in solvent E showed 2 : 3 : 4-tri-*O*-methylxylose, 2 : 3 : 5-tri-*O*-methylarabinose, and an unidentified sugar. Demethylation gave xylose, arabinose, and galactose.

Fraction 5. Chromatography of the syrup (34 mg.) which had $[\alpha]_D$ +30° (*c* 0·59) in solvent E, showed 2 : 3 : 4 : 6-tetra-*O*-methylgalactose and 2 : 3 : 5-tri- and di-*O*-methylarabofuranose. The presence of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose was shown by conversion into the aniline derivative, m. p. and mixed m. p. 179—180°.

Fraction 6. The syrup (17 mg.) had $[\alpha]_D$ −35° (*c* 0·29) and chromatography showed a main component with R_G 0·83, giving a brown stain (and yellow fluorescence in ultraviolet light) with aniline oxalate (cf. 3 : 5-di-*O*-methyl-L-arabinose, R_G 0·83). Demethylation gave arabinose and traces of galactose and mannose. Paper ionophoresis showed that 3 : 4 : 6-tri-*O*-methylmannose (R_G 0·82) and 2 : 5- (R_G 0·85) and 3 : 5-di-*O*-methylarabinose (R_G 0·83) could be readily distinguished in mixtures; fraction 6 showed mainly 3 : 5-di-*O*-methylarabinose with small amounts of the other two sugars.

Fraction 7. The syrup (230 mg.), which had $[\alpha]_D$ −24° (*c* 0·42), was shown by chromatography and ionophoresis to contain approximately equal amounts of 2 : 5- and 3 : 5-di-*O*-methylarabinose. Attempts to separate the two components by gradient elution from charcoal containing borate buffer (pH 10) with butan-2-one [11] were unsuccessful. The major portion (160 mg.) was fractionated by elution from Amberlite resin IRA-400 (borate form) with 0·5M-boric acid.[12] Although much sugar was irreversibly absorbed on the resin, two ionophoretically pure fractions 7*a* (40 mg.) and 7*b* (10 mg.) were obtained. Fraction 7*a* was identified as 2 : 5-di-*O*-methyl-L-arabinose by conversion into 2 : 5-di-*O*-methyl-L-arabonamide, m. p. 122° and mixed m. p. 123—124°. Fraction 7*b* was identified as 3 : 5-di-*O*-methyl-L-arabinose by conversion into 3 : 5-di-*O*-methyl-L-arabonolactone, m. p. 65° and mixed m. p. (with sample m. p. 69—71°) 67—69°.

Fraction 8. Chromatography and ionophoresis showed the syrup (80 mg.) to contain 2 : 5- and 3 : 5-di-*O*-methylarabinose, 3 : 4 : 6-tri-*O*-methylmannose, and a trace of 2 : 3 : 4-tri-*O*-methylgalactose. Attempts to separate the components by chromatography on charcoal containing borate buffer [11] failed.

Fraction 9. The syrup (174 mg.), which had $[\alpha]_D$ +72° ⟶ +119° (*c* 0·42), contained two components, R_G 0·65 and 0·70 respectively, present in approximately equal quantities and indistinguishable from 2 : 3-di-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methylgalactose. Demethylation gave arabinose and galactose. The syrup was converted into the corresponding mixture of aldonamides, from which 2 : 3-di-*O*-methyl-L-arabonamide readily crystallised, m. p. 154° and mixed m. p. 153—154°.

Fraction 10. The chromatographically pure syrup (299 mg.) had $[\alpha]_D$ +109° (*c* 0·51) and R_G 0·70. Recrystallisation from acetone–ether afforded 2 : 3 : 4-tri-*O*-methyl-D-galactose hydrate, m. p. 66—67°. The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-galactosylamine had m. p. and mixed m. p. 161—162°.

Fraction 11. Chromatography of the syrup (59 mg.), which had $[\alpha]_D$ +88° (*c* 0·33), showed 2 : 3 : 4-tri-*O*-methylgalactose (R_G 0·70) and a trace of an unknown sugar (R_G 0·52). The main component was identified by conversion into 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 164—165°.

Fraction 12. Chromatography of the syrup (155 mg.) showed a main component, R_G 0·60, and a small amount of 2 : 3 : 4-tri-*O*-methylgalactose. Demethylation gave arabinose and a small amount of galactose. The optical rotation, $[\alpha]_D$ +118° (*c* 0·38), indicated that the major component was a di-*O*-methyl-L-arabopyranose. Chromatographic examination in solvent F differentiated the sugar from 2 : 3- and 3 : 4-di-*O*-methylarabinose, and ionophoresis showed 3 : 4-di-*O*-methylarabinose to be absent. The syrup (60 mg.), when heated with ethanolic aniline, afforded an aniline derivative, m. p. 129—130° and mixed m. p. (with sample, 139—140°) 125—126°, whose *X*-ray powder photograph was identical with that of 2 : 4-di-*O*-methyl-*N*-phenyl-L-arabinosylamine.

Fraction 13. Chromatography of the syrup (280 mg.), which had $[\alpha]_D$ +55° (*c* 0·29), showed a major component (R_G 0·49), a small amount of tri-*O*-methylgalactose, and a trace of a sugar (R_G 0·32) suspected of being 2-*O*-methylarabinose. Demethylation gave galactose and a trace of arabinose. Paper ionophoresis showed three components, a small component travelling at the same rate as 2 : 3-di-*O*-methylgalactose, an unidentified component in traces [(?) 2-*O*-

methylarabinose], and the main component stationary (cf. 2 : 4-di-*O*-methylgalactose). Chromatography of the products of periodate oxidation showed 2 : 4-di-*O*-methylgalactose (R_F 0·37, unchanged), and small amounts of oxidation products with R_F 0·66 (grey), 0·78 (brown), and 0·15 (bright yellow). The first two oxidation products are formed from 2 : 3-di-*O*-methylgalactose, and the third from 2-*O*-methylaldoses (probably from 2-*O*-methylarabinose). The major component was identified as 2 : 4-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 206—208°.

Fraction 14. The chromatographically pure sugar (393 mg.) crystallised from acetone–water and had m. p. and mixed m. p. (with 2 : 4-di-*O*-methyl-D-galactose monohydrate) 97—99° and $[\alpha]_D$ +133° ⟶ +89° (equil.) (*c* 0·54) (Found: OMe, 27·8. Calc. for $C_8H_{16}O_6,H_2O$: OMe, 27·4%). The derived 2 : 4-di-*O*-methyl-*N*-phenyl-D-galactosylamine had m. p. and mixed m. p. 213—214°.

Fraction 15. Chromatography of the syrup (109 mg.) showed 2 : 4-di-*O*-methylgalactose, a sugar with R_G 0·36, and a trace of (?) 2-*O*-methylarabinose. Demethylation gave galactose, mannose, and a trace of arabinose. Ionophoresis showed di-*O*-methylgalactose (stationary), and a second sugar moving faster than 2- and 3-*O*-methylmannose. Separation of the syrup (100 mg.) on cellulose with solvent G gave fractions 15*a* and 15*b*. Fraction 15*a* contained 2 : 4-di-*O*-methylgalactose and (?) 2-*O*-methylarabinose (R_G 0·32) and gave galactose and arabinose on demethylation. Chromatography of the products of periodate oxidation [2] showed unchanged di-*O*-methylgalactose and a component, R_F 0·15 (bright yellow), formed from 2-*O*-methylaldoses. Fraction 15*b* was identified as 4-*O*-methyl-D-mannose by conversion into 4-*O*-methyl-D-mannonolactone, m. p. and mixed m. p. 159—160°.

Fraction 16. Chromatography of the syrup (47 mg.) showed 4-*O*-methylmannose (R_G 0·36) and a second sugar in smaller amount (R_G 0·32). The optical rotation, $[\alpha]_D$ +43° ⟶ +37° (*c* 0·33) (cf. 4-*O*-methyl-D-mannose, $[\alpha]_D$ +32° ⟶ +22°), and methoxyl content (Found: OMe, 17·3. Calc. for $C_7H_{14}O_6$: OMe, 16·0. Calc. for $C_6H_{12}O_5$: OMe, 18·9%) were consistent with those of a mixture of 4-*O*-methyl-D-mannose and a mono-*O*-methyl-L-arabinose. Furthermore, chromatography showed periodate oxidation products with R_F 0·60 (brown) and 0·15 (yellow) identical with those from 4-*O*-methyl-D-mannose and 2-*O*-methylaldoses. Attempts to characterise the sugar by conversion into 4-*O*-methyl-D-glucosazone failed, although an impure fraction, m. p. 135—136°, was shown by circular paper chromatography to contain the desired compound together with a second component.

Fraction 17. Chromatography showed the syrup (84 mg.) to contain 2 : 4-di-*O*-methylgalactose, 4-*O*-methylmannose, and 2-*O*-methylgalactose.

Fraction 18. The chromatographically pure sugar (431 mg.; R_G 0·25) crystallised from glacial acetic acid and had m. p. and mixed m. p. (with 2-*O*-methyl-D-galactose) 146—147° and $[\alpha]_D$ +64° ⟶ +91° (equil.) (*c* 0·37). After recrystallisation from acetone–water the sugar had m. p. 154°.

Examination of Acidic Components.—Acidic fractions (F) and (19) (as barium salts), containing a trace of 2-*O*-methylgalactose, were combined and dissolved in water, barium ions were removed by passage through Amberlite resin IR-120(H), and the solution was concentrated to a syrup (376 mg.). The mixture of acids was refluxed with methanolic 1·3% hydrogen chloride (50 ml.) for 6 hr. The product, after neutralisation with silver carbonate and concentration, was dissolved in formaldehyde dimethyl acetal (40 ml.), lithium aluminium hydride (0·2 g.) was added, and the solution was refluxed for 2 hr. Excess of hydride was destroyed by water, the acetal layer was separated, the aqueous layer was taken to dryness, and the residue was extracted with chloroform and acetone. The combined organic extracts were concentrated to a syrup (330 mg.) which was hydrolysed with N-hydrochloric acid (30 ml.) for 6 hr. at 100° to give, after neutralisation and concentration, a syrupy mixture of sugars (230 mg.). Separation of the methylated sugars on cellulose (50 × 2·5 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (1 : 1), saturated with water, as eluant, gave nine fractions. Fraction *a* (21 mg., R_G 0·85) was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 134—135°. Fraction *b* (19 mg.; R_G 0·72 and 0·58) contained 2 : 3 : 4-tri-*O*-methylgalactose and 2 : 3-di-*O*-methylglucose (major component). Fraction *c* (47 mg.; R_G 0·58) was identified as 2 : 3-di-*O*-methyl-D-glucose by chromatography of the sugar and its periodate oxidation products [R_F 0·73 (bright yellow) and 0·63 (brown), ionophoresis, and by conversion into 2 : 3-di-*O*-methyl-D-gluconophenylhydrazide, m. p. 173—174° and mixed m. p. (with sample, m. p. 168—169°) 169—171°.

Fraction *d* (11 mg.; R_G 0·58 and 0·54) contained 2 : 3-di-*O*-methylglucose and a trace of an unidentified sugar. Fraction *e* (9 mg.; R_G 0·49) was identified as 2 : 4-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 212—214°. Fraction *f* (10 mg.; R_G 0·49 and 0·36) contained a mixture of 2 : 4-di-*O*-methylgalactose and 4-*O*-methylmannose. Fraction *g* (20 mg.; R_G 0·36) was identified as 4-*O*-methyl-D-mannose by chromatography of the sugar and its periodate oxidation product [R_F 0·60 (brown)], ionophoresis, and by conversion into 4-*O*-methyl-D-mannonolactone, m. p. and mixed m. p. (with sample of m. p. 161—162°) 150—151°. Fraction *h* (13 mg.; R_G 0·32) contained at least two components giving periodate oxidation products having R_F 0·60 (brown) and 0·15 (bright yellow). Fraction *j* (8 mg.; R_G 0·25) was identified as 2-*O*-methyl-D-galactose by chromatography of the sugar and its periodate oxidation product R_F 0·23 (bright yellow), and as the crystalline sugar, m. p. and mixed m. p. 139—140°.

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115. *The Constitution of Larch ε-Galactan.*

By G. O. Aspinall, E. L. Hirst, and Else Ramstad.

Hydrolysis of methylated larch ε-galactan yields 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 2 : 3 : 4-tri-*O*-methyl-D-galactose, and 2 : 4-di-*O*-methyl-D-galactose in approximately equal quantities, together with smaller amounts of 2 : 3 : 4-tri-*O*-methyl-L-arabinose, 2 : 5-di-*O*-methyl-L-arabinose, 2 : 4 : 6-tri-*O*-methyl-D-galactose, and 2-*O*-methyl-D-galactose. Partial acid hydrolysis of the polysaccharide affords two disaccharides, 6-*O*-β-D-galactopyranosyl-D-galactose and 3-*O*-β-D-galactopyranosyl-D-galactose, and a series of higher oligosaccharides. Partial acid hydrolysis of the fragment remaining after degradation of the periodate-oxidised polysaccharide with phenylhydrazine affords 3-*O*-β-D-galactopyranosyl-D-galactose, and only small amounts of the second disaccharide. It is concluded (i) that the larch polysaccharide is a highly branched arabogalactan in which the majority of L-arabinose residues are accommodated as 3-*O*-β-L-arabopyranosyl-L-arabofuranosyl side-chains, and (ii) that the framework of the molecule consists of chains of 1 : 3-linked β-D-galactopyranose residues, the majority of which carry side-chains containing an average of two 1 : 6-linked β-D-galactopyranose residues.

It has been shown previously [1] that the water-soluble ε-galactan from European larch wood (*Larix decidua*) is a complex polysaccharide containing D-galactose and L-arabinose residues in the approximate ratio of 6 : 1. Evidence was presented which indicated that the material examined was a mixture, the main component being a galactan containing only minimal quantities of arabinose residues, and there also was present either an arabogalactan or an araban and a second galactan. Hydrolysis of the methylated polysaccharide derived from the main component gave equimolecular proportions of 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, and 2 : 4-di-*O*-methyl-D-galactose, indicating a highly branched molecule for which several possible repeating units could be suggested. More recently Jones [2] has isolated 3-*O*-β-L-arabopyranosyl-L-arabinose from the products of mild acid hydrolysis, indicating the presence in the polysaccharide of some arabinose residues in the less common pyranose form. We now report the results of a further investigation of this material.

ε-Galactan, isolated from larch sawdust by aqueous extraction, gave on hydrolysis galactose (85%) and arabinose (12%). No evidence of heterogeneity in this sample was found by Heidelberger [3] in a study of the precipitation reactions of the polysaccharide with various pneumococcal sera. Furthermore, an ultracentrifugal examination, kindly carried out by Dr. C. T. Greenwood, indicated the presence of only one molecular species. This latter result is in contrast to the findings of Mosimann and Svedberg,[4] whose ultracentrifugal measurements indicated the presence of two distinct components in the ε-galactan from European larch.

ε-Galactan was converted into its fully methylated derivative, but we could find no evidence of heterogeneity of the type encountered in the earlier investigation.[1] Hydrolysis of the methylated polysaccharide gave 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, and 2 : 4-di-*O*-methyl-D-galactose in approximately equimolecular proportions, together with smaller amounts of 2 : 3 : 4-tri- and 2 : 5-di-*O*-methyl-L-arabinose, and 2-*O*-methyl-D-galactose. Since the physical constants found for the tri-*O*-methyl-D-galactose did not correspond exactly to those of pure 2 : 3 : 4-tri-*O*-methyl-D-galactose, a search was made for other trimethyl ethers of galactose. Careful fractional crystallisation of the aniline derivatives afforded a small quantity of the aniline derivative of 2 : 4 : 6-tri-*O*-methyl-D-galactose. The proportions of the two trimethyl ethers were estimated by determining the formaldehyde formed on periodate oxidation of the derived hexitols; the 2 : 3 : 4-isomer yields formaldehyde whereas the 2 : 4 : 6-isomer does not. The results indicated the presence

in the mixture of 2 : 3 : 4- (92%) and 2 : 4 : 6-tri-*O*-methyl-D-galactose (8%). Since this work was completed Jones and Perry [5] have reported the use of a similar procedure for the estimation of the relative proportions of mixtures of methylated sugars.

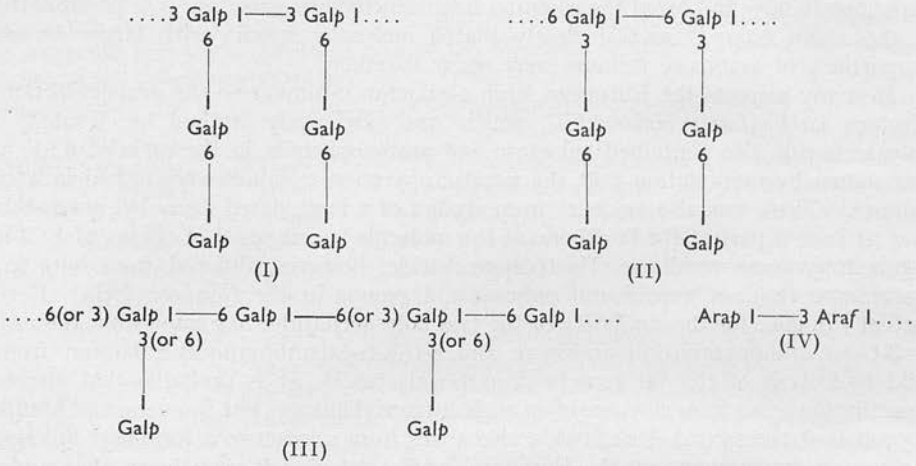
These results are in general accord with the previous findings [1] in respect of the main framework of galactose residues. The small quantity of galactose residues linked solely through $C_{(1)}$ and $C_{(3)}$ were not previously detected. Although the 2-*O*-methyl-D-galactose may have arisen from incomplete methylation of the polysaccharide, the absence of other monomethyl ethers of galactose suggests that this sugar may have structural significance, arising from a small proportion of doubly-branched galactose residues. 2 : 3 : 4-Tri-*O*-methyl-L-arabinose and 2 : 5-di-*O*-methyl-L-arabinose were isolated in approximately equimolecular amounts. In the absence of more than trace amounts of other arabinose derivatives it is clear that these sugars must represent fragments of an arabogalactan, and that on this evidence there is no indication of the presence in larch wood of polysaccharides composed solely of arabinose residues. Only traces of 2 : 3 : 5-tri-*O*-methylarabinose could be detected amongst the products of hydrolysis of the methylated polysaccharide and it is doubtful if terminal arabofuranose units are present to any significant extent in the polysaccharide. Since 3-*O*-β-L-arabopyranosyl-L-arabinose has been isolated from the products of partial acid hydrolysis of the polysaccharide,[2] it is now certain that the majority of arabinose residues are accommodated in 3-*O*-β-L-arabopyranosyl-L-arabofuranosyl side-chains, the furanose linkages being more easily cleaved on acid hydrolysis. Although this disaccharide has been isolated as an acid reversion from L-arabinose,[6] it is accompanied under these conditions by two other arabinose-containing disaccharides, neither of which was detected in the larch polysaccharide hydrolysate. Furthermore, in this instance, the disaccharide is destroyed when the hydrolysis is prolonged, whereas under the extreme conditions employed when acid reversion products are isolated in large amount [6] it is probable that an equilibrium is established between monomer and the various polymeric products.

On the basis of the methylation results various structures may be advanced for the repeating unit of the polysaccharide. Structures (I), (II), and (III) are representative of those containing an ordered arrangement of galactose residues; these structures do not take into account the mode of attachment of arabinose residues in side-chains (IV) to galactose. A partial distinction between the various possible structures has been made after the application of Barry's method of degradation [7] to the polysaccharide.

The periodate-oxidised ε-galactan was degraded by treatment with phenylhydrazine and acetic acid, and the products were separated into a mixture of compounds of low molecular weight and a polysaccharide residue. The products of low molecular weight were fractionated on alumina, and glyoxal bisphenylhydrazone and glycerosazone were isolated crystalline. Traces of galactosazone and arabinosazone were detected chromatographically, but the structural significance of these trace amounts is doubtful in view of the slightly acid conditions used in the degradation. Chromatography of the products of partial acid hydrolysis of the polysaccharide residue showed galactose, arabinose, and a series of galactose-containing oligosaccharides. The various structures represented by formula (III) can now be rejected since these contain only isolated galactose residues unattacked by periodate. The polysaccharide must contain, therefore, a resistant backbone of galactose residues with the majority of galactose residues, which are attacked by periodate, being accommodated in the side-chains of the molecular structure (I or II). A distinction between structures (I) and (II) has been made on the basis of evidence obtained from a study of the oligosaccharides isolated on partial acid hydrolysis of ε-galactan and of the periodate-oxidised polysaccharide after degradation with phenylhydrazine.

The oligosaccharides derived from ε-galactan on partial hydrolysis were separated on charcoal–Celite by elution with water containing increasing quantities of ethanol. Two

disaccharides, readily distinguishable by paper chromatography, were isolated pure. The 1 : 6-linked galactobiose was differentiated from the 1 : 3-linked isomer by the fact that the latter only gave formaldehyde on periodate oxidation. Galactobiose I had an optical rotation indicative of a β-linkage, and the structure of the disaccharide was established since hydrolysis of the methylated derivative afforded 2 : 3 : 4 : 6-tetra- and 2 : 3 : 4-tri-*O*-methyl-D-galactose. Galactobiose II, isolated crystalline, had similar physical constants to those recorded for 3-*O*-β-D-galactopyranosyl-D-galactose, isolated from the graded hydrolysis of *Acacia pycnantha* gum.[8] The mode of linkage in the disaccharide was confirmed by the isolation of 2 : 3 : 4 : 6-tetra- and 2 : 4 : 6-tri-*O*-methyl-D-



galactose on hydrolysis of the methylated derivative. Three galactose-containing trisaccharides were also isolated, and, although insufficient quantities of these compounds were available for full structural determinations, preliminary experiments provided evidence of their probable structures. Galactotriose I, isolated crystalline, gave only 6-*O*-galactosylgalactose and galactose on partial acid hydrolysis and is probably *O*-D-galactopyranosyl-(1 ⟶ 6)-*O*-D-galactopyranosyl-(1 ⟶ 6)-D-galactose. Galactotriose II gave both the 1 : 3- and the 1 : 6-linked galactobiose on partial hydrolysis, but since the derived glycitol gave galactose and no reducing disaccharide on partial hydrolysis, it is probable that the branched trisaccharide is 3 : 6-di-*O*-galactopyranosylgalactose. Galactotriose III gave 3-*O*-galactosylgalactose and galactose on partial hydrolysis and is probably *O*-galactopyranosyl-(1 ⟶ 3)-*O*-galactopyranosyl-(1 ⟶ 3)-galactose. Such a 1 : 3-linked galactotriose could only arise from a polysaccharide in which the repeating unit (I) is an important part of the molecular structure.

The degraded polysaccharide, remaining after treatment of the periodate-oxidised ε-galactan with phenylhydrazine and acetic acid, was subjected to graded acid hydrolysis, the products were fractionated on charcoal, and the disaccharide fractions were examined. 3-*O*-β-D-Galactopyranosyl-D-galactose, isolated crystalline, was the major component of this fraction and only small quantities of the 1 : 6-linked disaccharide were detected chromatographically. It follows from this observation that the backbone of the polysaccharide, resistant to attack by periodate, is composed of chains of 1 : 3-linked β-D-galactopyranose residues. The significance of the small amount of 6-*O*-galactosylgalactose detected in this experiment is not yet clear. It is possible that the backbone of the molecule may contain a small proportion of 1 : 6-linkages. Alternatively, if the arabinose containing side-chains are attached to the outer chains of the molecule, *e.g.*, through position 3 of galactose, some galactose residues in the outer part of the molecular structure would be unattacked by periodate and would give rise to the 1 : 6-linked disaccharide on partial hydrolysis of the degraded polysaccharide.

We can now summarise the main conclusions from these experiments. The ε-galactan from European larch is a highly branched arabogalactan in which the majority of arabinose residues are accommodated as 3-*O*-β-L-arabopyranosyl-L-arabofuranosyl side-chains (IV) linked to the framework of galactose residues. There is no evidence yet for the presence in larch wood of a polysaccharide composed solely of arabinose residues. The arrangement of galactose residues is best represented by the repeating unit (I), in which each D-galactopyranose residue in a 1 : 3-linked chain carried through position 6 an average of two 1 : 6-linked D-galactopyranose residues. In this respect, the molecular structure is strikingly similar to that now known to be present in gum arabic.[9] Although these experiments have indicated the essential homogeneity of ε-galactan, it is possible that, as in the xylan group,[10] several closely-related molecular species with larger or smaller proportions of arabinose residues may occur together.

In many respects the European larch ε-galactan is similar to the arabogalactan from Western larch (*Larix occidentalis*), which was extensively studied by White.[11] This polysaccharide also contained galactose and arabinose units in the ratio of 6 : 1, and it was shown by methylation that the D-galactopyranose residues were linked in a similar manner. There was also evidence from studies of a methylated degraded polysaccharide that at least a part of the backbone of the molecule is composed of chains of 1 : 3-linked D-galactopyranose residues. The polysaccharide, however, differed from ours in that L-arabinose residues were found only as end groups in the furanose form. Recently, further evidence for the similarity of the two polysaccharides has come from the isolation of 3-*O*-β-L-arabopyranosyl-L-arabinose and 6-*O*-β-D-galactopyranosylgalactose from the mild hydrolysis of the Western larch arabogalactan.[12] It is probable that the former disaccharide arises from cleavage of an arabofuranosyl linkage, but Bouveng and Lindberg [12] suggest that the second disaccharide also arises from scission of a furanosyl linkage. In preliminary experiments on the European larch ε-galactan it was shown that under the hydrolysis conditions required to release 3-*O*-arabopyranosylarabinose, appreciable quantities of the 1 : 6-linked galactobiose were also formed. Furthermore, attempts to prepare an arabinose-free galactan by selective hydrolysis under mild conditions failed as extensive breakdown of the polysaccharide to galactose-containing oligosaccharides occurred before all the arabinose residues were removed. Since we could find no evidence for the presence in the polysaccharide of galactofuranose residues, it is clear that in this case the rates of hydrolysis of arabofuranosides and galactopyranosides are not markedly different.

Experimental

Paper chromatography was on Whatman No. 1 filter paper, the following solvent systems (v/v) being used: (A) butan-1-ol–ethanol–water (40 : 11 : 19); (B) ethyl acetate–pyridine–water (10 : 4 : 3); (C) benzene–ethanol–water (169 : 47 : 15; upper layer); (D) butan-1-ol–formic acid–water (500 : 115 : 385; upper layer); (E) butan-2-one saturated with water.

The larch ε-galactan was prepared at the Forest Products Research Laboratory, Princes Risborough, by extraction of the sawdust with water, purification by passage through columns of Amberlite resins IR-120 and IRA-400, and precipitation from aqueous solution with ethanol. The polysaccharide yielded on hydrolysis galactose (85%) and arabinose (12%), the proportions of the sugars being estimated by Hirst and Jones's [13] method. Preliminary chromatographic studies showed that when the ε-galactan was heated at 100° with 0·01N-hydrochloric acid, arabinose, galactose, 3-*O*-arabopyranosylarabinose, and galactobiose I were released. On prolonged hydrolysis the arabinose-containing disaccharide disappeared and there was no indication of its formation under these conditions as an acid reversion product. An attempt was made to prepare a degraded galactan devoid of arabinose residues by mild acid hydrolysis (0·01N-hydrochloric acid at 100°), but even after heating for 20 hr. the precipitable degraded polysaccharide still contained arabinose residues, and galactose and galactose-containing oligosaccharides could be detected in the supernatant liquid.

Methylation of ε-Galactan.—ε-Galactan was methylated extensively with methyl sulphate

and sodium hydroxide to give a methylated polysaccharide, $[\alpha]_D^{18}$ −49° (*c* 1·2 in chloroform), $[\alpha]_D^{18}$ −27° (*c* 1·1 in methanol) [Found: OMe, 44·1; dimethylaraban, 5·2% (based on the yield of furfuraldehyde on distillation with 12% hydrochloric acid under standard conditions [14])]. Fractionation of the methylated polysaccharide failed to yield materials differing significantly in dimethylaraban content.

Hydrolysis of Methylated ε-Galactan and Separation of Methylated Sugars.—The methylated polysaccharide (10 g.) was suspended in 2N-sulphuric acid (150 ml.) and kept at room temperature until dissolution was complete. Water (150 ml.) was added and the solution was warmed slowly so that the methylated polysaccharide remained in solution and was then heated at 100° for 7 hr. The cooled solution was neutralised with barium carbonate and on concentration gave a syrupy mixture of methylated sugars (9·8 g.). A portion (5 g.) of the hydrolysate was separated on cellulose (80 × 3·5 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water as eluant to give ten fractions; a further fraction was obtained by elution of the cellulose with water.

Analysis of hydrolysate of methylated ε-galactan.

Fraction	Material eluted (g.)	R_G in solvent A *	Paper chromatography sugar *	Sugar given after demethylation *
1	0·006	0·96	2 : 3 : 5-trimethylarabinose	arabinose
2	0·437	0·96 (*t*)	2 : 3 : 5-trimethylarabinose	arabinose (*t*)
		0·90	2 : 3 : 4 : 6-tetramethylgalactose	galactose
3	0·701	0·90	2 : 3 : 4 : 6-tetramethylgalactose	—
4	0·033	0·90	2 : 3 : 4 : 6-tetramethylgalactose	—
		0·86	2 : 5-dimethylarabinose	—
		0·83	2 : 3 : 4-trimethylarabinose	—
5	0·211	0·86	2 : 5-dimethylarabinose	arabinose
		0·83	2 : 3 : 4-trimethylarabinose	
6	0·022	0·86	2 : 5-dimethylarabinose	arabinose
		0·83	2 : 3 : 4-trimethylarabinose	galactose
		0·72	trimethylgalactose	
7	0·970	0·72	trimethylgalactose	galactose
8	0·057	0·72	trimethylgalactose	galactose
		0·59 (*t*)	unknown sugar	arabinose (*t*)
		0·52 (*t*)	unknown sugar	
9	1·315	0·46	2 : 4-dimethylgalactose	—
10	0·260	0·30	2-methylgalactose	—
11	0·101	—	galactose (*t*), arabinose (*t*) + methylated uronic acid	—

* *t* = trace

Identification of Sugars from Hydrolysis of Methylated ε-Galactan.—*Fraction* 2. The syrup consisted almost entirely of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, paper chromatography indicating only a trace of tri-*O*-methylarabinose. The syrup crystallised on nucleation and after recrystallisation from ether–light petroleum had m. p. and mixed m. p. 68° (Found: OMe, 52·5. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). The sugar was further characterised as 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 192°.

Fraction 3. The sugar, after recrystallisation, had m. p. and mixed m. p. (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose) 68°, $[\alpha]_D^{19}$ +142° (5 min.) ⟶ +117° (3 hr., const.) (*c* 1·1 in water) (Found: OMe, 53·1. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%), and its identity was confirmed by conversion into the aniline derivative, m. p. and mixed m. p. 192°.

Fraction 5. The syrup had OMe, 41·0%, $[\alpha]_D^{20}$ +82° (*c* 1·2 in water), and chromatography showed two components travelling at the same rates as 2 : 3 : 4-tri-*O*-methyl and 2 : 5-di-*O*-methyl-L-arabinose, with traces of methylated galactoses. The mixture of sugars (270 mg.), obtained from a separate experiment, was separated on filter sheets, solvent C being used, to give fractions 5*a* (111 mg.) and 5*b* (154 mg.), each of which contained only traces of the second component. Fraction 5*a*, $[\alpha]_D^{20}$ +120° (*c* 2·2 in water) (Found: OMe, 46·1. Calc. for $C_8H_{16}O_5$: OMe, 48·4%), was identified by conversion into 2 : 3 : 4-tri-*O*-methyl-L-arabonophenylhydrazide, m. p. 157° (Jones [2] quotes m. p. 159°). Fraction 5*b*, $[\alpha]_D^{18}$ −3° (*c* 1·5 in water) (Found: OMe, 33·9. Calc. for $C_7H_{14}O_5$: OMe, 34·8%), was identified by conversion into 2 : 5-di-*O*-methyl-L-arabonomide, m. p. 131°.

Fractions 4 *and* 6. The combined fractions were shown by chromatography to contain the same two sugars as fraction 5, together with smaller quantities of methylated galactoses. A portion (41 mg.), separated on filter sheets by using solvent C, yielded chromatographically pure 2 : 3 : 4-tri-*O*-methyl- (11 mg.) and 2 : 5-di-*O*-methyl-arabinose (22 mg.).

Fraction 7. The crystalline sample, which travelled on the chromatogram at the same rate as 2 : 3 : 4- and/or 2 : 4 : 6-tri-*O*-methyl-D-galactose, had $[\alpha]_D^{20}$ +125° (5 min.) ⟶ +104° (2 hr., const.) (*c* 1·1 in water) and after dehydration at 60° over phosphoric oxide under reduced pressure had $[\alpha]_D^{20}$ +109° (equil.) (*c* 1·1 in water) [Found (after dehydration): OMe, 41·3. Calc. for $C_9H_{18}O_6$: OMe, 41·9%]. Recrystallisation from acetone–ether–light petroleum gave a substance, m. p. (unaltered on further recrystallisation) 56°, $[\alpha]_D^{20}$ +139° (5 min.) ⟶ +109° (2 hr., const.) (*c* 1·1 in water) (Found: OMe, 38·2. Calc. for $C_9H_{18}O_6,H_2O$: OMe, 38·7%). {2 : 3 : 4-Tri-*O*-methyl-D-galactose monohydrate is reported to have m. p. 80°, $[\alpha]_D$ +152° ⟶ +114° (in water) and 2 : 4 : 6-tri-*O*-methyl-D-galactose to have m. p. 104—105°, $[\alpha]_D$ +124° ⟶ +93° (in water).} A sample (100 mg.) was dehydrated and refluxed with ethanolic aniline for 5 hr.; recrystallisation of the product from acetone yielded the characteristic plates of 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 166° (a mixture with the characteristic needles of 2 : 4 : 6-tri-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. 171°, had m. p. 144°). Concentration of the mother liquor yielded a mixture, m. p. 143°, of needles and plates but it was not possible to separate the two components.

Fraction 9. The chromatographically pure sugar was recrystallised from acetone containing 1% of water and had m. p. and mixed m. p. (with 2 : 4-di-*O*-methyl-D-galactose monohydrate) 102°, $[\alpha]_D^{20}$ +136° (5 min.) ⟶ +86° (2 hr., const.) (*c* 1·1 in water) (Found: OMe, 27·1. Calc. for $C_8H_{16}O_6,H_2O$: OMe, 27·2%). The identity of the sugar was confirmed by conversion into 2 : 4-di-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 215°.

Fraction 10. The sugar (recrystallised from glacial acetic acid) had m. p. and mixed m. p. (with 2-*O*-methyl-D-galactose) 148—149°, $[\alpha]_D^{20}$ +53° (5 min.) ⟶ +85° (2 hr., const.) (*c* 0·9 in water), and the derived 2-*O*-methyl-*N*-phenyl-D-galactosylamine had m. p. 163°.

Fraction 11. The main component behaved chromatographically as a methylated uronic acid with low mobility in neutral solvents but having R_G 0·84 in solvent D and giving a characteristic cherry-red colour with aniline oxalate.

*Re-examination of Tri-*O*-methylgalactose Fraction.*—Recrystallisation of the tri-*O*-methylgalactose fraction (from a separate experiment) from acetone–light petroleum afforded 2 : 3 : 4-tri-*O*-methyl-D-galactose hydrate, m. p. 73—76° (some sintering from 65°). Tri-*O*-methylgalactose (0·6 g.; anhydrous) was heated with aniline (0·3 ml.) in ethanol (20 ml.) for 1 hr. After removal of solvent the crystalline product was fractionally crystallised from acetone and acetone–light petroleum. The first fractions were composed solely of the aniline derivative (plates), m. p. and mixed m. p. 163—165°, of 2 : 3 : 4-tri-*O*-methyl-D-galactose. Subsequent fractions contained two crystalline forms and mechanical separation of the needles, followed by two recrystallisations from acetone–light petroleum, afforded the aniline derivative (needles), m. p. 166—167°, mixed m. p. (with sample, m. p. 178°) 169—171°, of 2 : 4 : 6-tri-*O*-methyl-D-galactose ; the m. p. was depressed on admixture with the aniline derivative of 2 : 3 : 4-tri-*O*-methyl-D-galactose.

*Estimation of the Relative Proportions of Tri-*O*-methylgalactoses.*—Methyl ethers of D-galactose were converted into the corresponding methyl ethers of D-galactitol. The sugar (200 mg.) in water (10 ml.) was added to potassium borohydride (60 mg.) in water (5 ml.). The solution stood for 14 hr. at room temperature, excess of borohydride was destroyed by the addition of acetic acid, the solution was de-ionised by passage through columns of ion-exchange resins, Amberlite IRA-400 and Zeo-Karb 225, and concentrated to a crystalline residue. These materials were used without further purification in the periodate oxidation experiments. 2 : 4-Di-*O*-methyl-D-galactose afforded 2 : 4-*di*-O-*methyl*-D-*galactitol*, which after recrystallisation from ethanol–light petroleum had m. p. 133—134°, $[\alpha]_D^{20}$ +16° (*c* 0·3 in water) (Found: OMe, 30·1. $C_8H_{18}O_6$ requires OMe, 29·5%). The mixture of tri-*O*-methylgalactoses afforded the corresponding mixture of galactitols, recrystallisation from ethanol–light petroleum giving 2 : 3 : 4-*tri*-O-*methyl*-D-*galactitol*, m. p. 119°, $[\alpha]_D^{20}$ +6° (*c* 0·8 in water) (Found: OMe, 42·0. $C_9H_{20}O_6$ requires OMe, 41·5%).

The formaldehyde formed on periodate oxidation of the methylated hexitols was determined with chromotropic acid by O'Dea and Gibbons's method.[15] 2 : 4-Di-*O*-methyl-D-galactitol gave theoretical amounts of formaldehyde (cf. D-glucose). The formaldehyde formed

on oxidation of the mixture of tri-*O*-methyl-D-galactitols corresponded to the presence therein of 92% of the 2 : 3 : 4-isomer, and, by difference, of 8% of the 2 : 4 : 6-isomer.

Degradation of Periodate-oxidised ε-Galactan with Phenylhydrazine.—The polysaccharide (1·7 g.) was oxidised with sodium metaperiodate solution (120 ml.; 0·2M) for 144 hr. (consumption of periodate was complete, corresponding to the uptake of 8·7 moles of periodate per 6 residues of galactose and 1 residue of arabinose). The solution was treated with lead acetate to remove iodate and periodate, and then with dilute sulphuric acid to precipitate excess of lead. The solution of oxygalactan (160 ml.) was treated with phenylhydrazine (3·5 ml.) in 10% acetic acid (10 ml.), and the precipitate was washed with water and dried to a yellow powder (1·5 g.). The oxygalactan phenylhydrazine derivative was suspended in ethanol (40 ml.), phenylhydrazine (5 ml.) in glacial acetic acid (8 ml.) and water (15 ml.) was added, and the mixture was refluxed for 4 hr. Removal of the ethanol under reduced pressure yielded crystals A (1·7 g.) which were separated and washed. The filtrate was extracted with benzene and ether to give a dark red solution B, and concentration of the aqueous solution yielded a red gum C (0·6 g.).

The crystalline precipitate A was dissolved in benzene and adsorbed on alumina (80 g.). Elution with benzene (250 ml.) gave fraction Ai (1·0 g.) which on recrystallisation from benzene yielded glyoxal bisphenylhydrazone, m. p. and mixed m. p. 168°. Elution with 1 : 1 benzene–ether (350 ml.) gave fraction Aii (0·1 g.), recrystallisation from benzene yielding first *N*-acetylphenylhydrazine, m. p. and mixed m. p. 128°, and then glycerosazone, m. p. and mixed m. p. 130°. Elution with ether (175 ml.) gave fraction Aiii (0·25 g.), which on recrystallisation from benzene yielded *N*-acetylphenylhydrazine. Elution with 9 : 1 ethanol–water gave fractions Aiv (0·3 g.) (containing *N*-acetylphenylhydrazine), Av (0·04 g.), and Avi (0·01 g.). Circular-paper chromatography showed fraction Av to contain glycerosazone and a trace of galactosazone, and fraction Avi to contain glycerosazone, arabinosazone, and galactosazone.

The benzene and ether extracts (B) yielded more *N*-acetylphenylhydrazine on concentration (fraction Bi). The residual syrup was dissolved in benzene and adsorbed on alumina (100 g.). Elution with ether (500 ml.) yielded fraction Bii (0·05 g.), shown by chromatography to contain only glyoxal bisphenylhydrazone. Elution with 9 : 1 ethanol–water gave fractions Biii (1·06 g.) and Biv (0·07 g.). Fraction Biii on recrystallisation from benzene–light petroleum yielded *N*-acetylphenylhydrazine and chromatographic examination of the mother liquor showed glyoxal bisphenylhydrazone and glycerosazone. Chromatographic examination of fraction Biv showed arabinosazone, galactosazone, and material which did not move on the chromatogram. Hydrolysis of fraction Biv yielded galactose and arabinose.

The red gum (C) consisted of the backbone of the polysaccharide remaining unattacked by periodate, hydrolysis of which gave arabinose and galactose. Partial acid hydrolysis yielded a series of galactose-containing oligosaccharides.

Partial Acid Hydrolysis of ε-Galactan.—ε-Galactan (10 g.) was heated (boiling-water bath) in 0·2N-sulphuric acid for 3 hr. The cooled solution was neutralised with Amberlite resin IR-4B, concentrated to 100 ml., and poured into ethanol (400 ml.). The precipitated degraded polysaccharide was separated at the centrifuge and concentration of the supernatant liquid gave a syrup (D) (4·46 g.). The degraded polysaccharide was re-hydrolysed by N-sulphuric acid (200 ml.) for 1 hr. at 100° and gave syrup (E) (3·76 g.) and a gum (1·8 g.) insoluble in ethanol–water (4 : 1). Chromatography showed that syrups (D) and (E) contained similar mixtures of galactose, arabinose, and a series of oligosaccharides. The combined syrups (8·21 g.) were dissolved in water and poured on charcoal–Celite (1 : 1; 300 g.). Elution with water gave fraction 1 (6·12 g.) containing galactose and arabinose. Elution with water containing 2·5% of ethanol gave fraction 2 (368 mg.) containing a disaccharide (galactobiose I), R_{Gal} 0·40 in solvent B, and traces of other sugars. Elution with water containing 5% of ethanol gave fraction 3 (260 mg.) containing a disaccharide (galactobiose II), R_{Gal} 0·60 in solvent B. Elution with water containing 7·5% of ethanol gave fraction 4 (40 mg.), containing an oligosaccharide having R_{Gal} 0·18 and traces of other sugars, and fraction 5 (160 mg.), having a major component with R_{Gal} 0·25. Elution with water containing 10% of ethanol gave several small fractions (total, 58 mg.) containing mixtures of oligosaccharides having R_{Gal} 0·25, 0·15, and 0·10, which were not examined. Elution with water containing 15% of ethanol gave fraction 6 (128 mg.), having a major component with R_{Gal} 0·30.

Examination of Oligosaccharides.—Fraction 2. Chromatographically pure galactobiose I was isolated by separation on filter sheets with solvent B; the syrup had $[\alpha]_D^{17}$ +41° (*c* 1·18 in

water). Oxidation of the disaccharide (12 mg.) with periodate [16] gave no formaldehyde. The major portion of the syrup (247 mg.) was dissolved in water (5 ml.), and methyl sulphate (1 ml.) and sodium hydroxide (1·5 ml., 30%) were added dropwise during 2 hr. Two more additions of methyl sulphate (7 ml.) and sodium hydroxide (10 ml., 30%) were made during a period of 6 hr. The reaction was completed by heating the solution on the boiling-water bath for 30 min., and the methylated disaccharide (243 mg.) was isolated by continuous extraction with chloroform for 12 hr. Hydrolysis of a sample (1 mg.) of the methylated disaccharide gave only tetra- and tri-*O*-methylgalactose. Methylated galactobiose I (93 mg.) was heated with N-hydrochloric acid (10 ml.) at 100° for 4 hr., and, after neutralisation with silver carbonate, gave a syrup (68 mg.) which was separated on a filter sheet with solvent E yielding fractions *a* (32 mg.) and *b* (25 mg.). Fraction *a* was characterised as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose and fraction *b* as 2 : 3 : 4-tri-*O*-methyl-D-galactose by conversion into the aniline derivatives, m. p. and mixed m. p. 192—193°, and m. p. 159—160° and mixed m. p. (with sample of m. p. 163—165°), 160—163° respectively.

Fraction 3. Galactobiose II crystallised readily and after recrystallisation from ethanol–water had m. p. 175—177°, $[\alpha]_D^{17}$ +78° (10 min.) ⟶ +63° (120 min., equil.) (*c* 0·67 in water). Hirst and Perlin [8] record m. p. 159—160°, $[\alpha]_D$ +62° (in water) for 3-*O*-β-D-galactopyranosyl-D-galactose monohydrate. Periodate oxidation [16] of a sample (12 mg.) gave formaldehyde, identified as the dimedone compound, m. p. 188—190°. Galactobiose II (100 mg.) was methylated as described above to give the methylated disaccharide (106 mg.). Hydrolysis of methylated galactobiose II (106 mg.) with N-hydrochloric at 100° for 4 hr., followed by neutralisation with silver carbonate, gave a syrup (75 mg.) which was separated on a filter sheet with solvent E yielding fractions *c* (34 mg.), *d* (25 mg.), and *e* (7 mg.). Fraction *c* was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose and fraction *d* as 2 : 4 : 6-tri-*O*-methyl-D-galactose by conversion into the aniline derivatives, m. p. and mixed m. p. 185—187°, and m. p. and mixed m. p. 168—169°, respectively. Fraction *e* contained di-*O*-methylgalactose, probably arising from incomplete methylation of the disaccharide.

Fraction 4. A chromatographically pure sugar, having R_{Gal} 0·18 in solvent B, m. p. 157—160°, $[\alpha]_D^{18}$ +16° (equil.) (*c* 0·38 in water), crystallised from ethanol–water. Partial acid hydrolysis of the sugar gave galactose, galactobiose I, and unchanged sugar.

Fraction 5. A sample of the major component, R_{Gal} 0·25 in solvent B, was separated. The sugar gave on partial acid hydrolysis galactose, galactobiose I, galactobiose II, and unchanged sugar. Partial hydrolysis of the derived glycitol (borohydride reduction) gave galactose and no other reducing sugars.

Fraction 6. A sample of the major component, R_{Gal} 0·30 in solvent B, was separated chromatographically. Partial acid hydrolysis of the sugar gave galactose, galactobiose II, and unchanged sugar, and partial hydrolysis of the derived glycitol gave galactose and galactobiose II.

Partial Acid Hydrolysis of Gum (C).—Gum (C), prepared from ε-galactan (6·5 g.) by phenylhydrazine degradation of the periodate-oxidised polysaccharide as described above, was heated with N-sulphuric acid (100 ml.) at 100° for 1 hr. The cooled solution was neutralised with Amberlite resin IR-4B and extracted with ether, the extract concentrated, and ethanol (2 vol.) added. A small precipitate was removed at the centrifuge and concentration of the supernatant liquid gave a syrup (1·82 g.) which was dissolved in water and poured on charcoal–Celite (1 : 1; 100 g.). Elution with water gave a mixture (0·80 g.) of galactose and arabinose. Elution with water containing 2·5% of ethanol gave a syrup (33 mg.) containing galactose, arabinose, and a small amount of galactobiose I, R_{Gal} 0·40. Elution with water containing 5% of ethanol gave the crystalline galactobiose II (60 mg.), R_{Gal} 0·60, which after recrystallisation from ethanol–water had m. p. 176—179° and mixed m. p. 175—178°, $[\alpha]_D^{18}$ +78° (5 min.) ⟶ +62° (60 min., equil.) (*c* 0·51 in water).

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124. *Cereal Gums. Part III.* The Constitution of an Araboxylan from Barley Flour.*

By G. O. Aspinall and R. J. Ferrier.

A water-soluble polysaccharide from barley flour gave xylose (59%), arabinose (37%), and glucose (4%) on hydrolysis. Hydrolysis of the methylated polysaccharide gave 2 : 3 : 5-tri-*O*-methyl-L-arabinose (3 parts), 2 : 3-di-*O*-methyl-D-xylose (3 parts), a mixture (1 part) of 2- and 3-*O*-methyl-D-xylose, and D-xylose (1 part). It is concluded that the highly branched barley araboxylan contains chains of 1 : 4-linked β-D-xylopyranose residues to some of which terminal L-arabofuranose residues are attached through positions 2 and 3.

Barley gum, isolated from the grain by aqueous extraction, is a mixture of polysaccharides containing residues of glucose, xylose, and arabinose.[1] Preece and Mackenzie [1] isolated the major component, a β-glucan, by fractional precipitation from aqueous solution by addition of ammonium sulphate. It was shown in Part I [2] that this polysaccharide contains unbranched chains of β-D-glucopyranose residues with approximately equal proportions of 1 : 3- and 1 : 4-linkages. Other fractions from barley gum gave mainly xylose and arabinose on hydrolysis, but a pure pentosan was not obtained by this method. An indication that the barley pentosan is similar to that found in wheat flour [3] was obtained by Gilles, Meredith, and Smith [4] who isolated three components on fractionation of methylated barley gum: (*a*) a methylated araboxylan; (*b*) a methylated α-glucan; and (*c*) a methylated β-glucan. Hydrolysis of the methylated araboxylan gave 2 : 3 : 5-tri-*O*-methylarabinose (1 part), 2 : 3-di-*O*-methylxylose (12 parts), 2-*O*-methylxylose (4 parts), and xylose (2 parts). As part of a series of structural investigations of the polysaccharide components of barley,[2, 5, 6] we now report a more detailed study of the barley-flour araboxylan.

Barley gum, isolated from the flour by aqueous extraction at 40°,[1] was fractionated by addition of ammonium sulphate to the aqueous solution. Fractions rich in pentosan, but still containing appreciable quantities of β-glucan, were precipitated at high concentrations of ammonium sulphate. Acetylation of the mixture of polysaccharides, followed by fractional precipitation of the acetates from acetone solution by the addition of light petroleum, afforded substantially pure acetylated araboxylan. Hydrolysis of a sample of pentosan, regenerated from the acetate, gave xylose (59%), arabinose (37%), and glucose (4%).

The acetylated araboxylan was simultaneously deacetylated and methylated. Hydrolysis of a sample of the methylated polysaccharide and quantitative paper chromatography of the hydrolysate [7] showed the presence of tri-*O*-methyl- (41%), di-*O*-methyl- (36%), mono-*O*-methyl- (10%), and unsubstituted (13%) pentose. The mixture of methylated sugars obtained on hydrolysis of the methylated polysaccharide was fractionated on cellulose to give 2 : 3 : 5-tri-*O*-methyl-L-arabinose (39%), 2 : 3-di-*O*-methyl-D-xylose (36%), and D-xylose (14%), characterised as crystalline derivatives, and a mixture of mono-*O*-methylxyloses (11%). Paper ionophoresis showed that the mono-*O*-methylxylose fraction contained both the 2- and the 3-methyl ether, and the optical rotation of the syrupy mixture of sugars showed these to be present in the approximate ratio of 2 : 1. A portion of the mixture was converted into the corresponding mixture of methyl pyranosides, and the consumption of periodate, 0·76 mol., indicated that the 2- and the 3-methyl ether were present in the ratio of 3 : 1.

Several possible structures for the repeating unit of the polysaccharide may be put forward on the basis of these results, (I) and (II) being typical. In structure (I) the backbone of D-xylopyranose residues is linear and the L-arabofuranose residues are attached as single unit side-chains. In structure (II), on the other hand, the backbone of xylose residues is branched and the longer side-chains are terminated by arabofuranose residues.

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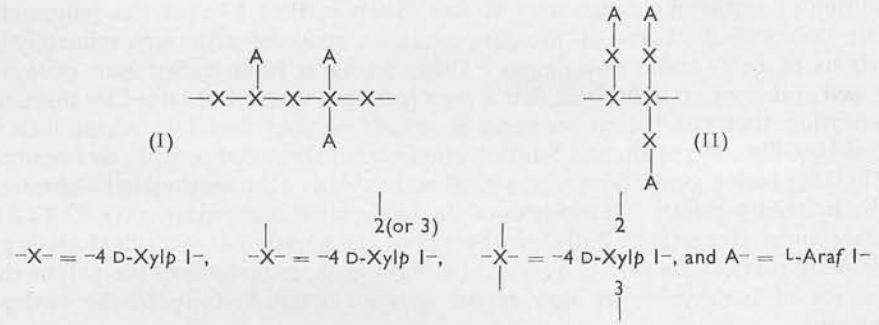
The following results provide evidence in favour of structure (I). The methylated araboxylan was heated with dilute aqueous methanolic hydrogen chloride under controlled conditions. The degraded methylated polysaccharide was separated from methylated sugars formed during the mild hydrolysis, and chromatographic examination of the sugars showed that about a quarter of the 2 : 3 : 5-tri-*O*-methylarabinose had been selectively removed, together with only a trace of di-*O*-methylxylose. The degraded methylated polysaccharide was remethylated and hydrolysed; quantitative estimation showed that 2 : 3 : 5-tri-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methylxylose were present in the hydrolysate in the ratio of 11 : 1. For an araboxylan of structure (I) mild hydrolysis of the methylated polysaccharide followed by remethylation would cause no significant increase in xylose end-groups. On the other hand, with an araboxylan of structure (II), these operations would yield a methylated degraded polysaccharide with an increased number of xylose end-groups, each terminal arabinose removed giving rise to a terminal xylose residue. Thus, removal of approximately one quarter of the arabinose residues



followed by remethylation would result in the formation of methylated degraded araboxylan containing arabinose and xylose end-groups in the proportion of 3 : 1. It follows from the observed ratio of arabinose to xylose end-groups of 11 : 1 that the majority of arabinose residues in the barley araboxylan must be attached directly to the backbone of xylose residues as in structure (I).

It is clear from these results that the barley-flour araboxylan is similar to other polysaccharides of the xylan group [8] in containing chains of 1 : 4-linked β-D-xylopyranose residues to which are attached single-unit L-arabofuranoses ide-chains. The polysaccharide resembles most closely the araboxylans from wheat [3] and rye [9] flour, differing only in that some of the singly-branched xylose residues carry arabinose residues attached to $C_{(2)}$ whereas in other xylans arabinose residues are linked to $C_{(2)}$ only in the case of doubly-branched xylose residues carrying a substituent at $C_{(3)}$ also. It is of particular interest that within the general structural pattern common to the various xylans from land plants [8] the araboxylans from cereal grains form a group of substances which differ from the typical xylans of lignified tissues in containing a higher proportion of arabinose but no glucuronic acid residues often found in the latter group. Barley-flour araboxylan and barley-husk hemicellulose [6] provide an example of polysaccharides from the same plant which differ in detailed chemical structure and probably also in biological function.

Experimental

Paper chromatography was carried out on Whatman No. 1 filter paper, with the upper layers of the following solvent systems (v/v): (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (B) butan-1-ol–ethanol–water (4 : 1 : 5); (C) benzene–ethanol–water (169 : 47 : 15). Paper ionophoresis was in borate buffer at pH 10. Optical rotations were observed at 18° ± 2°. Methylated sugars were demethylated with hydrobromic acid.[10]

Extraction and Fractionation of Barley Gum.—Barley (Carlsberg variety, harvested in 1953) was passed through an automatic polishing machine to remove husks, and the grain was ground to a fine flour. Barley flour (8 kg.), previous extracted with boiling ethanol–water (4 : 1) to

inactivate enzymes and remove soluble sugars, waxes, and colouring matter, was extracted with water at 40° according to Preece and Mackenzie's [1] procedure and gave barley gum (14 g.; 0·18% of the grain). Precipitation of the gum from aqueous solution by the addition of ammonium sulphate afforded fractions (1) (8 g.; mainly glucan precipitated with 20% ammonium sulphate) and (2) (5·7 g.; precipitated with 30% and 40% ammonium sulphate). Fraction (2) had $[\alpha]_D$ −125° ± 5° (*c*, 0·2 in N-sodium hydroxide) and quantitative chromatography of the hydrolysate [11] showed xylose (49%), arabinose (32%), and glucose (19%). Fraction (2) (5 g.) was dispersed in formamide (65 ml.), and pyridine (195 ml.) was added and then acetic anhydride (100 ml.) slowly with stirring during 4 hr. The mixture was stirred for 2 days at room temperature and poured into water (2 l.) to give acetylated polysaccharide (6·4 g.), which was exhaustively extracted with acetone leaving a residue (0·21 g.). Addition of light petroleum (b. p. 60—80°; 250 ml.) to the acetone extract (500 ml.) gave acetate (A) (3·8 g.), $[\alpha]_D$ −107° (*c*, 1·0 in pyridine) [Found: OAc, 34·3%], and concentration of the supernatant liquid gave acetate (B) (2·2 g.), $[\alpha]_D$ −39° (*c* 1·0 in pyridine) [Found: OAc, 35·5%]. A sample of acetate (A) (0·5 g.) was deacetylated by Zemplen and Pacsu's [12] method to give polysaccharide (0·266 g.), $[\alpha]_D$ −104° (*c* 0·6 in N-sodium hydroxide), hydrolysis and quantitative chromatography [11] showing xylose (59%), arabinose (37%), and glucose (4%). A sample of acetate (B) was hydrolysed directly and quantitative chromatography showed xylose (29%), arabinose (27%), and glucose (44%).

Methylation.—Acetylated polysaccharide (A) (2·9 g.) was methylated by successive treatments with methyl sulphate and sodium hydroxide, and fractional precipitation of the product from chloroform by the addition of light petroleum (b. p. 60—80°) gave fractions (C) (1·177 g.), $[\alpha]_D$ −140° (*c* 0·5 in chloroform) (Found: OMe, 37·3%), and (D) (0·412 g.), $[\alpha]_D$ −134° (*c* 0·5 in chloroform) (Found: OMe, 37·7%). Samples of (C) and (D) were hydrolysed, and chromatography showed tri-*O*-methylarabinose, di- and mono-*O*-methylxylose, and xylose; small amounts of tri-*O*-methylglucose were also detected in the hydrolysate from (D), but not in this experiment in that from (C). Further methylation of fraction (C) gave methylated araboxylan (0·94 g.), $[\alpha]_D$ −137° (*c* 0·6 in chloroform) (Found: OMe, 38·2%). Hydrolysis of a sample of methylated araboxylan, and quantitative chromatography [7] of the hydrolysate showed the relative amounts of pentose sugars to be: tri-*O*-methylarabinose, 4·1 parts, di-*O*-methylxylose, 3·6 parts, mono-*O*-methylxylose, 1 part, and xylose 1·3 parts.

Hydrolysis of Methylated Araboxylan and Separation of Methylated Sugars.—Methylated araboxylan (0·9 g.) was hydrolysed successively with boiling methanolic 3% hydrogen chloride (100 ml.) for 3·5 hr. and 0·5N-hydrochloric acid (100 ml.) at 100° for 3 hr. After neutralisation with silver carbonate, concentration gave a syrupy mixture of methylated sugars (0·72 g.) which was fractionated on cellulose (40 × 2 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water, and butan-1-ol partly saturated with water, as eluants, to give eight fractions.

Fraction 1. The syrup (197 mg.) had $[\alpha]_D$ −35° (*c* 0·5 in water) (Found: OMe, 47·9. Calc. for $C_8H_{16}O_5$: OMe, 48·4%), and was chromatographically indistinguishable from 2 : 3 : 5-tri-*O*-methyl-L-arabinose in solvents B and C. Demethylation gave arabinose. The sugar was characterised by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 133—135°.

Fraction 2. Chromatography in solvent B showed the syrup (28 mg.) to contain 2 : 3 : 5-tri-*O*-methylarabinose and tri-*O*-methylglucose in the approximate ratio of 3 : 1. Chromatography in solvent C showed that a trace of 2 : 3 : 4-tri-*O*-methylxylose was also present. Demethylation gave arabinose, glucose, and a trace of xylose.

Fraction 3. Chromatography in solvent B showed the syrup (43 mg.) to contain tri-*O*-methylglucose and 2 : 3-di-*O*-methylxylose. The optical rotation ($[\alpha]_D$ +44°, in water) was consistent with the presence of tri-*O*-methylglucose (2 : 3 : 6- and 2 : 4 : 6-trimethyl ethers have $[\alpha]_D$ +70°, in water [13]) and 2 : 3-di-*O*-methyl-D-xylose ($[\alpha]_D$ +23° in water [15]) in the ratio 1 : 1·2.

Fraction 4. The chromatographically pure syrup (154 mg.) had $[\alpha]_D$ +25° (*c* 0·7 in water) (Found: OMe, 33·5. Calc. for $C_7H_{14}O_5$: OMe, 35·0%), and gave xylose on demethylation. The sugar was characterised as 2 : 3-di-*O*-methyl-D-xylose by conversion into the aniline derivative, m. p. and mixed m. p. 123—124°.

Fraction 5. The syrup (45 mg.) travelled on the chromatogram in solvent B at the same rate as 2- and 3-*O*-methyl-D-xylose, and ionophoresis showed both methyl ethers to be present (Found: OMe, 18·6. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). Demethylation gave xylose. The optical rotation ($[\alpha]_D$ +29°, in water) was consistent with the presence of 2-*O*-methyl-D-xylose

($[\alpha]_D$ +17°) [15] and 3-*O*-methyl-D-xylose ($[\alpha]_D$ +35°) [16] in the ratio 2 : 1. The syrupy mixture of methyl pyranosides, prepared by refluxing the syrup with dry methanolic hydrogen chloride, consumed 0·76 mol. of periodate (spectrophotometric determination [17]).

Fraction 6. The syrup (9 mg.) contained an unknown sugar *a* (R_G 0·30 in solvent B) and xylose in the approximate ratio of 2 : 1. Hydrolysis of sugar *a* gave di- and mono-*O*-methylxylose in about equal amounts. In subsequent calculations 3 mg. of each were added to the quantities of xylose, mono-, and di-*O*-methylxylose isolated in pure fractions.

Fraction 7. Chromatographically pure D-xylose (45 mg.) crystallised and had m. p. and mixed m. p. 139—142°, $[\alpha]_D$ +22° (equil.) (in water).

Fraction 8. Chromatography showed the syrup (23 mg.) to contain xylose and a number of slower-moving components. The syrup was hydrolysed and quantitative estimation indicated the presence in the mixture of residues of xylose (13 mg.) and mono- (4 mg.) and di-*O*-methylxylose (6 mg.). In subsequent calculations these quantities were added to the appropriate fractions.

The quantities of sugars isolated from the hydrolysis of the methylated araboxylan indicate their presence in the following molar percentages: 2 : 3 : 5-tri-*O*-methyl-L-arabihose (39%), 2 : 3-di-*O*-methyl-D-xylose (36%), mono-*O*-methyl-D-xylose (11%), and D-xylose (14%).

Partial Hydrolysis of Methylated Araboxylan.—Methylated araboxylan (fraction D, 56 mg.) was dissolved in methanol (5 ml.), 0·2N-hydrochloric acid (5 ml.) was added, and the mixture was refluxed for 4 hr. The solution was neutralised with Amberlite resin IR-4B, filtered, and freeze-dried. The residue was exhaustively extracted with boiling light petroleum (b. p. 60—65°), and chromatographic examination of the extract (4·5 mg.) showed 2 : 3 : 5-tri-*O*-methylarabinose and a trace of 2 : 3-di-*O*-methylxylose. The residual methylated polysaccharide was remethylated with methyl iodide and silver oxide and yielded methylated degraded araboxylan (36 mg.) (Found: OMe, 38·9%). The methylated polysaccharide was hydrolysed as described previously, the tri-*O*-methylpentose fraction was resolved chromatographically by solvent C, and quantitative estimation by Pridham's [18] method showed 2 : 3 : 5-tri-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methylxylose to be present in the ratio of 11 : 1. On treatment with *p*-anisidine hydrochloride the sugars gave products having absorption maxima at 390 and 500 mμ, respectively, and it was shown that at these wavelengths and for weights of sugar from 10—40 μg. the absorption was directly proportional to the quantity of sugar.

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211. *The Hemicelluloses of European Larch* (Larix decidua). *Part I. The Constitution of a Xylan.*

By G. O. Aspinall and J. E. McKay.

Larch hemicellulose fractions are composed of residues of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose, and 4-*O*-methyl-D-glucuronic acid. From methylation and other experiments it is concluded that there is present a xylan containing unbranched chains of *ca.* 100 1 : 4-linked β-D-xylopyranose residues with every fifth or sixth residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue linked through position 2, and a smaller proportion of xylose residues carrying, on position 3, side-chains terminated by L-arabofuranose residues.

Larches are the only common deciduous conifers. They differ chemically from other coniferous woods in containing a much higher proportion of galactose-containing polysaccharides. The galactans are easily extracted with water and those from European [1] and Western [2] larches have been extensively studied. It was of interest to extend the investigations to the alkali-soluble hemicelluloses and this paper describes the structure of a xylan from European larch wood (*Larix decidua*).

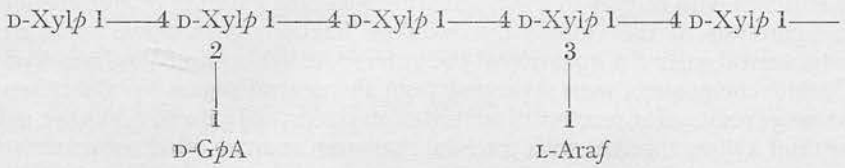
Larch sawdust was extracted with hot water to remove ε-galactan and was then partially delignified with acidified sodium chlorite solution. The resulting holocellulose was extracted with 1% and 4% aqueous sodium hydroxide, to give hemicellulose fractions I and II, and with 10% sodium hydroxide to give fraction III (precipitated on acidification of the extract) and fraction IV (precipitated on subsequent addition of acetone). Hydrolysis and chromatography showed the various fractions to be composed of the same sugar residues but in different proportions. Fraction I contained mainly xylan and galactan, fraction II xylan together with appreciable amounts of glucomannan, and fractions III and IV mainly glucomannan with smaller amounts of xylan. Fractions I and II were fractionated by precipitation from water by ammonium sulphate,[3] to give fractions enriched with respect to xylan but still containing galactan or glucomannan.

Since hydrolysis of the various hemicellulose fractions gave acidic components in addition to neutral sugars, a quantity of the unfractionated hemicellulose was hydrolysed and the acidic components were separated from the neutral sugars by absorption on an anion-exchange resin. The mixture of neutral sugars contained galactose, glucose, mannose, arabinose, and xylose, together with traces of rhamnose and two other sugars with greater chromatographic mobility. One of the last two sugars was tentatively identified by chromatography as 3-*O*-methylxylose. As far as we are aware this sugar has not previously been found to occur naturally, although the 2-methyl ether has recently been identified as a constituent of plum leaf hemicellulose.[4] The mixture of acidic components was eluted from the anion-exchange resin, and the three main components were separated by chromatography on filter sheets. The first fraction was identified by chromatography and optical rotation as 4-*O*-methyl-D-glucuronic acid. The second fraction was an *O*-(4-*O*-methyl-α-D-glucuronosyl)-D-xylose since the aldobiouronic acid had a high positive rotation ($[\alpha]_D$ +99°) and reduction of the derived methyl ester methyl glycoside with potassium borohydride followed by hydrolysis gave 4-*O*-methyl-D-glucose and D-xylose. The third fraction was probably an aldotriouronic acid since hydrolysis gave the aldobiouronic acid and xylose.

One of the xylan-rich fractions was methylated in the usual way and fractionation of the methylated polysaccharide gave a methylated xylan containing only traces of a methylated hexosan. Hydrolysis of the methylated xylan furnished 2 : 3-di-*O*-methyl-D-xylose and a methylated aldobiouronic acid, together with small amounts of 2 : 3 : 5-tri-*O*-

methylarabinose and 2 : 3 : 4-tri-*O*-methyl- and 2- and 3-*O*-methyl-xylose, which were only detected chromatographically. The structure of the methylated aldobiouronic acid was shown by the following experiments to be 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-α-D-glucuronosyl)-D-xylose. The acidic disaccharide was converted into the methyl ester methyl glycoside which was reduced with lithium aluminium hydride. Hydrolysis of part of the partially methylated disaccharide gave 2 : 3 : 4-tri-*O*-methylglucose and 3-*O*-methyl-xylose. The remaining material was remethylated and hydrolysis of the fully methylated disaccharide gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and 3 : 4-di-*O*-methyl-D-xylose. From the quantities of the major components isolated from the hydrolysis of the methylated polysaccharide it may be concluded that this xylan contains chains of 1 : 4-linked β-D-xylopyranose residues with approximately every fifth xylose residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue attached as a side-chain to position 2.

In order to obtain more detailed information regarding the fine structure of larch xylans a second series of methylation studies was carried out. The methylated xylan with uronic acid residues present as the sodium salts was readily separated from the methylated polysaccharides without uronic acid residues. The acidic groups were reduced with lithium aluminium hydride, and the reduced methylated xylan was remethylated. Hydrolysis of the methylated reduced xylan afforded 2 : 3 : 4 : 6-tetra- and 2 : 3 : 4-tri-*O*-methyl-D-glucose, 2 : 3 : 5-tri-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 2- and 3-*O*-methyl-D-xylose in the approximate molar ratio of 6 : 12 : 5 : 1 : 72 : 20 : 5. The tetra- and tri-*O*-methyl-D-glucose arise from the D-glucuronic acid residues in the xylan, not all tri-*O*-methyl-D-glucose residues being completely remethylated after the reduction. From the previous results it is clear that the 3-*O*-methyl-D-xylose arises from branching points in the main chain to which the uronic acid groups are attached. Since 3-*O*-methyl-D-xylose and the mixture of methyl ethers of D-glucose are present in approximately equimolecular amounts, and since 2-*O*-methyl-D-xylose and 2 : 3 : 5-tri-*O*-methyl-L-arabinose also occur in approximately the same molar proportions, it is probable that the 2-methyl ether arises from branch points to which terminal L-arabofuranose residues are attached. The accompanying partial structure for the xylan indicates the main features of the molecule.



(D-Xyl*p* = D-xylopyranose, D-G*p*A = 4-*O*-methyl-D-glucuronic acid, and L-Ara*f* = L-arabofuranose)

It is not possible on the present evidence to indicate whether the L-arabofuranose residues are attached directly to the backbone of xylose residues as shown or whether 1 : 4-linked D-xylose residues are interposed with the arabinose residues terminating a longer side-chain. The former alternative is more probable as other xylans (from, *e.g.*, wheat straw [5] and barley husks [6]) are known to contain L-arabofuranose residues directly linked to the backbone of xylose residues.

A molecular-weight determination by the isothermal-distillation method (by the courtesy of Dr. C. T. Greenwood) gave a value of 18,000 ± 500 (degree of polymerisation, 107 ± 3) for the methylated xylan (as methyl ester). This value, taken together with the value of one non-reducing xylose end group per *ca.* 120 sugar residues, suggests that the backbone of xylose residues is unbranched. It is concluded, therefore, that this xylan fraction contains chains of 1 : 4-linked β-D-xylopyranose residues with, on the average, every fifth or sixth residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue linked through $C_{(2)}$. In addition, a small proportion (*ca.* 4%) of L-arabofuranose residues

are present as integral parts of the xylan; these are present as non-reducing end-groups and are probably attached to the backbone through $C_{(3)}$ of xylose residues. In the first methylated xylan fraction, which we examined, the proportion of arabinose residues was very low (<1%) and it is probable that some of the xylan chains carried no arabinose residues. It is clear that in larch wood, as in monocotyledenous plants,[7] several closely related xylans occur side by side.

Several wood xylans have now been examined and all are characterised by the presence of 4-*O*-methyl-D-glucuronic acid residues attached as side-chains to D-xylose by 1 : 2-linkages. The proportions of uronic acid groups are in general somewhat higher in the xylans from soft woods (15—20%) (Western hemlock,[8] Norway spruce,[9] and larch) than in those from hard woods (8—15%) European [10] and North American [11] beech, birch,[12] and aspen [13]). Some of these xylans (Western hemlock, aspen, and larch) also contain a small proportion of L-arabofuranose residues. In contrast, the xylans from cereals [7] are, in general, characterised by a higher proportion of arabinose groups and a lower proportion of uronic acid groups. Despite considerable variations in detailed molecular architecture, however, it is not possible to draw a clear line of demarcation on structural grounds between the xylans from different lignified tissues.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 and 3MM filter papers with the following solvent systems (v/v): (A) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (B) ethyl acetate–pyridine–water (10 : 4 : 3); (C) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (D) benzene–ethanol–water (169 : 47 : 15, upper layer); (E) butan-1-ol–acetic acid–water (4 : 1 : 5, upper layer); (F) butan-2-one, saturated with water containing 1% of ammonia; (G) pentan-2-one, 75% saturated with water; (H) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer). Methylated sugars were demethylated with hydrobromic acid.[14] Optical rotations were observed at 18° ± 2°. Extractions and reactions involving the use of alkali were performed, as far as possible, under nitrogen. In typical extractions larch holocellulose samples (*ca.* 80 g.) were extracted with alkali (*ca.* 1 l.) in a ball-mill for 12 hr. at room temperature.

Extraction and Fractionation of Larch Hemicellulose.—Larch sawdust was extracted successively with boiling benzene and boiling methanol to remove fats and colouring material, and then with water at 90° to remove ε-galactan. The residual sawdust was delignified with acidified sodium chlorite solution according to Wise's procedure.[15] The resulting holocellulose was extracted three times each with 1%, 4%, and 10% sodium hydroxide solutions to give fraction I (6% by weight of the air-dried sawdust), II (4·6%), III (4·0%), and IV (5·0%). Fractions I and II were isolated by acidification of the alkaline extracts with acetic acid (to pH 4—5) and precipitation with an equal volume of acetone. Fraction III was precipitated from the 10% sodium hydroxide extract on acidification, and fraction IV was precipitated on addition of an equal volume of acetone to the supernatant liquid after removal of fraction III. Fraction I, by precipitation from aqueous solution on the stepwise addition of ammonium sulphate, gave a xylan-rich fraction I*a* (precipitated with 50% ammonium sulphate) and galactan-rich fraction I*b* and I*c* (precipitated with 30% and 60% ammonium sulphate). Refractionation of fractions I*a* failed to yield xylan free from galactan. Fraction II was refractionated in a similar way, giving fractions II*a* and II*b* (precipitated with 30 and 50% ammonium sulphate). After five reprecipitations with ammonium sulphate fraction II*b* gave xylan A (1·6%), which was still contaminated with glucomannan. Fraction III was separated into fractions III*a* (1·0%) and III*b* (3·0%), soluble and insoluble in 2% aqueous sodium hydroxide. Fraction III*b* was further separated into fractions III*c* (0·3%), insoluble in 10% sodium hydroxide, III*d* (2·0%), soluble in 10% sodium hydroxide and precipitated on acidification, and III*e* (0·7%), soluble in 10% sodium hydroxide and precipitated, after acidification and removal of III*d*, by the addition of acetone. Fraction IV was separated into fractions IV*a* (4·3%) and IV*b* (0·7%), soluble and insoluble in water.

Chromatography [16] in solvent B of the hydrolysate from xylan A (Found: uronic anhydride, 11·7%) showed xylose (46·5%), mannose + arabinose (18%), glucose (9%), and galactose (3%).

Fraction III*c* (0·5 g.) in acetic anhydride (5 ml.), acetic acid (5 ml.), and concentrated

sulphuric acid (0·28 ml.) was kept at room temperature for 7 days. The mixture was poured into ice-water (50 ml.), and the chloroform extract (2 × 25 ml.) was dried and concentrated. The resulting sugar acetates were deacetylated with barium methoxide in methanol and the sugars were examined by chromatography in solvent B. In addition to glucose and traces of mannose, xylose, and galactose, oligosaccharides having the same mobilities as cellobiose and cellotriose were detected. Fraction III*c* was probably a degraded form of cellulose which was extracted together with glucomannan (cf. Aspinall, Laidlaw, and Rashbrook [17]).

Samples of the various hemicellulose fractions were hydrolysed and the relative proportions of the sugars detected on paper chromatograms are indicated in the Table.

Hemicellulose fraction	Method of isolation	Paper chromatography					
		Man	Glu	Gal	A	Xyl	UA
I	Extrn. with 1% NaOH	+	+	+++	++	+++	++
I*a*	Ammonium sulphate	+	+	+	+	+++	++
I*b* and I*c*	Pptn.	—	—	+++	++	+	tr
II	Extrn. with 4% NaOH	++	+	+	+	+++	++
II*a*	Ammonium sulphate	+++	++	+	tr	+++	++
II*b*	Pptn.	++	+	+	tr	+++	++
III	Extrn. with 10% NaOH	+++	++	+	—	++	+
III*a*	Sol. in 2% NaOH	+++	++	+	—	+++	++
III*b*	Insol. in 2% NaOH	+++	++	+	—	+	tr
III*c*	Insol. in 10% NaOH	tr	+++	tr	—	tr	—
III*d*	Sol. in 10% NaOH	+++	++	tr	—	tr	—
III*e*	Sol. in 10% NaOH	+++	++	+	—	+	tr
IV	Extrn. with 10% NaOH	+++	++	+	—	++	+
IV*a*	Sol. in H_2O	+++	++	+	—	+++	++
IV*b*	Insol. in H_2O	+++	++	+	—	+	tr

(Man = mannose, Glu = glucose, Gal = galactose, A = arabinose, Xyl = xylose, and UA = uronic acid. tr = trace.)

Hydrolysis of Larch Hemicellulose and Examination of the Acidic Components.—Hemicellulose (fractions I and II; 30 g.) in N-sulphuric acid (700 ml.) was heated on the boiling-water bath for 13·5 hr. (constant rotation). The solution was neutralised with barium hydroxide and barium carbonate, then filtered, and the barium salts were washed with water (3 × 30 ml.). The combined filtrate and washings were freed from barium ions by passage through a column of Amberlite resin IR-120(H) and the acids were adsorbed on a column of Amberlite resin IR-4B (OH). The eluate was concentrated to a syrup (20 g.), and chromatography showed galactose, glucose, mannose, arabinose, and xylose together with traces of rhamnose and two faster-moving sugars. One of the last two sugars was chromatographically similar to 3-*O*-methyl-D-xylose, but distinct from the 2-methyl ether, and gave xylose on demethylation.

The acids were displaced from the resin with N-sulphuric acid, and the eluate was neutralised with barium carbonate, filtered, freed from barium ions with Amberlite resin IR-120(H), and concentrated to a syrup (1·2 g.). Chromatography in solvent A showed three main components having R_{xylose} 1·26, 0·69, and 0·17, and traces of sugars having R_{xylose} 1·60 (probably glucurone) and 0·30. Pure samples of the major acidic sugars were obtained by chromatography on filter sheets with solvent A. Fraction *a* (110 mg.) had $[\alpha]_D$ +83° (*c* 1·1 in H_2O) and was chromatographically indistinguishable from 4-*O*-methyl-D-glucuronic acid (R_{xylose} 1·26). Fraction *b* (231 mg.) had R_{xylose} 0·69 and $[\alpha]_D$ +99° (*c* 1·15 in H_2O). The acid was converted into the methyl ester methyl glycoside (185 mg.) which was treated with potassium borohydride (400 mg.) in water (10 ml.) for 18 hr. Excess of hydride was destroyed by the addition of acetic acid, and the solution was de-ionised by passage through columns of Amberlite resins IR-120(H) and IR-4B(OH) and concentrated to a syrup (140 mg.). The syrup (135 mg.) was hydrolysed with 0·8N-sulphuric acid (5 ml.) at 100° for 5 hr. After neutralisation with barium carbonate the resulting syrup (106 mg.) was separated on filter sheets with solvent H, to give fractions *d* (43 mg.) and *e* (35 mg.). Fraction *d* had $[\alpha]_D$ +54° (*c* 0·85 in H_2O) and was identified as 4-*O*-methyl-D-glucose by conversion into the phenylosazone, m. p. 149—152°, characterised by circular paper chromatography and by *X*-ray powder photography. Fraction *e* had $[\alpha]_D$ +20° (*c* 0·70 in H_2O) and was identified as D-xylose by conversion into the di-*O*-benzylidene dimethyl acetal, m. p. and mixed m. p. 210°. The acid fraction *c* (150 mg.) had R_{xylose} 0·17 and on hydrolysis gave xylose and the aldobiouronic acid having R_{xylose} 0·69.

Preparation of Methylated Xylan A, Hydrolysis, and Separation of Methylated Sugars.—

Xylan A (10 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide to give methylated polysaccharide (3·5 g.), $[\alpha]_D$ −51·5° (*c* 0·34 in $CHCl_3$) (Found: OMe, 40·1%). A sample of this material was hydrolysed and chromatography of the hydrolysate showed methylated hexoses in addition to methylated pentoses and acidic components. The methylated polysaccharide was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 60—65°) mixtures and three main fractions were obtained, (1) [0·40 g., soluble in chloroform–light petroleum (1 : 4); OMe, 45·0%], (2) [0·95 g., soluble in chloroform–light petroleum (1 : 3); OMe, 38·5%], and (3) [1·0 g., soluble in chloroform–light petroleum (3 : 7); OMe, 37·9%]. Samples of these fractions were hydrolysed and the hydrolysates were examined chromatographically, fraction 1 giving methylated hexoses and traces of methylated pentoses, and fractions 2 and 3 giving methylated pentoses together with acidic components and traces of methylated hexosis. Fractions 2 and 3 were combined, dissolved in chloroform, and precipitated by light petroleum, to give methylated xylan A (1·8 g.), $[\alpha]_D$ −61° (*c* 0·23 in $CHCl_3$) (Found: OMe, 38·1%).

Methylated xylan A (1·5 g.) was hydrolysed successively with boiling methanolic 5% hydrogen chloride (170 ml.) for 8 hr. and with 0·5N-hydrochloric acid (100 ml.) at 100° for 12·5 hr. The cooled solution was neutralised with silver carbonate, hydrogen sulphide was passed through the filtrate to precipitate silver salts, and the solution was concentrated to a syrup. The syrup was dissolved in water, the acidic components were converted into barium salts by treatment with barium carbonate, and the solution was taken to dryness. The resulting syrup was extracted with chloroform to give syrup A (1·01 g.) and an insoluble residue, which was dissolved in water, passed through a column of Amberlite resin IR-100(H) to remove barium ions, and concentrated to give acidic fraction B (139 mg.).

Syrup A (1·01 g.) was fractionated on cellulose (40 × 3 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later, 1 : 1) saturated with water, and butan-1-ol half saturated with water as eluants, to give six fractions. Fractions 1—3 contained only neutral sugars. Fractions 4 and 6 contained only acidic components; these were combined and after removal of barium ions with Amberlite resin IR-100(H) gave acidic fraction C (116 mg.). Fraction 5 contained neutral and acidic sugars.

Examination of Neutral Sugars.—Chromatography of fraction 1 (26·5 mg.) in solvent C showed only one component (R_G 0·95). Since the syrup did not crystallise a small sample was rehydrolysed and a second component (R_G 0·73 in solvent C) was observed. The remainder of the syrup was rehydrolysed and chromatography in solvent D showed three sugars, 2 : 3 : 5-tri-*O*-methylarabinose, 2 : 3 : 4-tri-*O*-methylxylose, and 2 : 3-di-*O*-methylxylose. The proportions of the sugars were estimated by Pridham's method,[18] and the result indicated the presence in the original syrup of tri-*O*-methylxylose (10 mg.), tri-*O*-methylarabinose (5·5 mg.), and methyl di-*O*-methylxyloside (11 mg.). Fraction 2 (20 mg.) contained tri-*O*-methylhexose (R_G *ca.* 0·80 in solvent C) and on demethylation gave mannose, glucose, and a trace of galactose. Fraction 3 (404 mg.) crystallised on being seeded with 2 : 3-di-*O*-methyl-β-D-xylose and had m. p. 78° and $[\alpha]_D$ +23° (equil.) (*c* 0·82 in H_2O) (Found: OMe, 35·1. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The identity of the sugar was confirmed by conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 122—123°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. 132°. Fraction 5 (32 mg.) contained mono-*O*-methylxylose and a small amount of an acidic component and had $[\alpha]_D$ +44° (*c* 1·43 in H_2O). The neutral sugars (28 mg.) were separated from the acid on a filter sheet by using solvent C, and paper ionophoresis showed 2- and some 3-*O*-methylxylose. The syrupy mixture of methyl pyranosides, prepared by refluxing the sugars with dry methanolic hydrogen chloide, consumed 0·20 mol. of periodate (spectrophotometric determination [19] carried out by Dr. R. J. Ferrier), showing that the 2- and the 3-methyl ether were present in the ratio of 1 : 4.

Examination of the Acidic Fractions.—Chromatography of acidic fractions B and C in solvent E showed both to contain a main component and a second component moving slightly more slowly. On vigorous hydrolysis both fractions gave 2 : 3 : 4-tri-*O*-methylglucuronic acid, mono-*O*-methylxylose, and a trace of 2 : 3-di-*O*-methylxylose. Fraction C (95 mg.) was refluxed with methanolic 1% hydrogen chloride (15 ml.) for 6 hr. The product, after neutralisation with silver carbonate, was dissolved in methylal (15 ml.), lithium aluminium hydride (100 mg.) was added, and the solution was refluxed for 2 hr. Excess of hydride was destroyed by water, and the methylal layer was separated and concentrated. The aqueous layer was taken to dryness and the residue was extracted with acetone. The solid residue was suspended in

water (25 ml.), shaken with Amberlite resin IR-100(H), taken to dryness, and extracted again with acetone. The combined extracts (70 mg.) were hydrolysed with 0·8N-hydrochloric acid (14 ml.) at 100° for 6 hr., and the hydrolysate (45 mg.) was separated on a filter sheet by using solvent E, to give fractions *a* (19 mg.) and *b* (7 mg.). Fraction *a* had $[\alpha]_D$ +50° (±3°) and was chromatographically identical with 2 : 3 : 4-tri-*O*-methyl-D-glucose. Fraction *b* was chromatographically and ionophoretically indistinguishable from 3-*O*-methyl-D-xylose and gave xylose on demethylation. Acidic fraction B (125 mg.), after conversion into methyl ester methyl glycoside, was reduced in a similar way, and the product was methylated with methyl iodide and silver oxide. The fully methylated disaccharide (95 mg.) was hydrolysed with 0·8N-hydrochloric acid (15 ml.) at 100° for 6 hr., and the hydrolysis products were separated on filter sheets with solvent E to give fractions *c* (53 mg.) and *d* (33 mg.). Fraction *c* had $[\alpha]_D$ +80° (±2°) and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 135—137°. Chromatography and ionophoresis of fraction *d* showed only 3 : 4-di-*O*-methylxylose, and the sugar was characterised by conversion into 3 : 4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 65·5—66·5°.

Preparation of Methylated Reduced Xylan B.—Larch hemicellulose (fraction II; 20 g.) was methylated with methyl sulphate and sodium hydroxide, and the crude methylated polysaccharide which was isolated was separated into fractions C and D, respectively soluble and insoluble in acetone. The insoluble residue (D) was dispersed in acetone–water (1 : 1), further acetone (3 vol.) was added, inorganic salts, which separated, were filtered off, the filtrate was taken to dryness, and the residue was separated into fractions E and F, respectively soluble and insoluble in boiling chloroform. Fractions C and E were combined, dissolved in chloroform, and precipitated by the addition of light petroleum. This precipitate (7·5 g.) was probably a mixture of methylated glucomannan and methylated xylan (in the acid form) since chromatography of the hydrolysate showed methylated hexoses, methylated pentoses, and acidic components. The chloroform-insoluble residue (F) was dispersed in ethanol–water (9 : 1), and addition of ether precipitated methylated xylan B (12·0 g.; as sodium salt). Chromatography of the hydrolysate showed methylated pentoses together with acidic components, but no methylated hexoses. Methylated xylan B (11·9 g.; as sodium salt) in acetone–water (400 ml., 1 : 1) was shaken with Amberlite resin IR-120(H) for 30 min., further acetone (200 ml.) being added to maintain solution. Acetone was removed from the filtrate under reduced pressure at 15°, the aqueous dispersion was extracted with chloroform, and the dried extract was concentrated and poured into light petroleum to give methylated xylan B (7·9 g., as free acid). The methylated xylan acid (7·9 g.) was treated with methyl iodide and silver oxide, giving the methylated xylan methyl ester (7·3 g.), $[\alpha]_D$ −52° (*c* 0·89 in $CHCl_3$) (Found: OMe, 39·3%). The methylated polysaccharide (7·1 g.) in refluxing tetrahydrofuran (330 ml.) was reduced by lithium aluminium hydride (1·4 g.) for 3 hr. After destruction of excess of hydride by water the mixture was taken to dryness and the methylated polysaccharide was isolated by extraction with acetone. After a second reduction with lithium aluminium hydride, the product was remethylated with methyl iodide and silver oxide, to give methylated reduced xylan B (5·2 g.), $[\alpha]_D$ −52° (*c* 0·40 in $CHCl_3$) (Found: OMe, 37·2%).

Hydrolysis of Methylated Reduced Xylan B and Separation of Methylated Sugars.—0·5N-Hydrochloric acid was added slowly to methylated reduced xylan B (4·5 g.) in boiling methanol (300 ml.). The rates of addition of acid and of distillation of methanol were adjusted so that complete solution was maintained, acid (650 ml.) being added during 10 hr. while 630 ml. of distillate were collected. Water (100 ml.) was added and the solution (N with respect to hydrochloric acid) was heated at 100° for 4 hr. (constant rotation). The cooled solution was neutralised with silver carbonate and concentrated to a syrup (4·8 g.) which was fractionated on cellulose (70 × 3 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water, and butan-1-ol half saturated with water as eluants, to give four fractions.

Fraction 1. Chromatography of the syrup (500 mg.) in solvents D and G showed 2 : 3 : 4 : 6-tetra-*O*-methylglucose, 2 : 3 : 5-tri-*O*-methylarabinose, and 2 : 3 : 4-tri-*O*-methylxylose. Since hydrolysis of a sample gave small amounts of di- and mono-*O*-methylxylose (presumably derived from methyl glycosides) the syrup (*ca.* 480 mg.) was rehydrolysed with 0·5N-hydrochloric acid (50 ml.) at 100° for 5 hr., and the hydrolysate (433 mg.) was refractionated on cellulose (80 × 2 cm.) with the same solvents, to give six fractions. Fraction 1*a* (23 mg.) had m. p. 72—75° and $[\alpha]_D$ +81° (equil.) (*c* 0·45 in H_2O) and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 134—135°.

Fraction 1*b* (178 mg.) contained 2 : 3 : 4 : 6-tetra-*O*-methylglucose and 2 : 3 : 5-tri-*O*-methylarabinose in the ratio of 0·73 : 1 (estimated by Pridham's method [18]). A sample (42 mg.) of 2 : 3 : 5-tri-*O*-methyl-L-arabinose, $[\alpha]_D$ −29° (*c* 0·42 in H_2O), was separated on filter sheets with solvent G and identified by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. 137—138°. Fraction 1*c* (27 mg.), $[\alpha]_D$ +19° (*c* 0·27 in H_2O), was chromatographically indistinguishable from 2 : 3 : 4-tri-*O*-methyl-D-xylose but attempts to prepare the aniline derivative failed. Fractions 1*d* (9 mg.), 1*e* (15 mg.), and 1*f* (9 mg.) were shown by chromatography to contain 2 : 3 : 4-tri-*O*-methylglucose, and 2 : 3-di- and mono-*O*-methylxylose respectively.

Fraction 2. The chromatographically pure syrup (492 mg.) (Found: OMe, 41·8. Calc. for $C_9H_{18}O_6$: OMe, 41·9%) had $[\alpha]_D$ +67° (*c* 0·82 in H_2O) and was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 144—145°.

Fraction 3. The sugar (2·325 g.) crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose and had m. p. 70—75° (Found: OMe, 34·5. Calc. for $C_7H_{14}O_5$: OMe, 34·9%). The sugar was identified by conversion into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 130—132°.

Fraction 4. Chromatography (solvent F) and ionophoresis of the syrup (730 mg.) disclosed 2- and 3-*O*-methylxylose. The optical rotation {$[\alpha]_D$ +23·0° (*c* 1·46 in H_2O)} of the syrup corresponded to that of a mixture of 2-*O*-methyl-D-xylose [20] ($[\alpha]_D$ +35·9°) and 3-*O*-methyl-D-xylose [10] ($[\alpha]_D$ +19·5°) in the ratio of 1 : 4. The periodate consumed (0·19 mol.) (spectrophotometric determination [19]) by the derived syrupy mixture of methyl pyranosides indicated that 19% of the 2-methyl ether was present in the mixture. The syrup (500 mg.) was refractionated on cellulose (40 × 3 cm.) with solvent R, to give three fractions. Fraction 4*a* (312 mg.), $[\alpha]_D$ +19° (*c* 0·69 in H_2O), was chromatographically and ionophoretically pure, and was identified as 3-*O*-methyl-D-xylose by conversion into the phenylosazone, m. p. and mixed m. p. 170—171°. Fraction 4*b* (45 mg.) contained mainly 3-*O*-methylxylose with small amounts of the 2-methyl ether. Fraction 4*c* (89 mg.), $[\alpha]_D$ +33° (*c* 0·36 in H_2O), contained mainly the 2-methyl ether with small amounts of the 3-methyl ether. 2-*O*-Methyl-D-xylose was identified by conversion into the aniline derivative, m. p. and mixed m. p. 125—126°.

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The Synthesis of 2-O-β-D-Xylopyranosyl-L-arabinose and its Isolation from the Partial Hydrolysis of Esparto Hemicellulose.

By G. O. ASPINALL and R. J. FERRIER.

The structure of 2-O-β-D-xylopyranosyl-L-arabinose has been confirmed by a synthesis involving a Koenigs-Knorr condensation of 2:3:4-tri-O-acetyl-α-D-xylopyranosyl bromide with benzyl 3:4-O-isopropylidene-β-L-arabopyranoside, followed by removal of protecting groups. The disaccharide has also been identified as one of the products of partial acid hydrolysis of esparto hemicellulose.

THE disaccharide, 2-O-β-D-xylopyranosyl-L-arabinose, was first isolated as a partial acid hydrolysis product from corn cob hemicellulose B,[1,2] and the mode of linkage was established by methylation.[1] We have recently isolated the same compound from the partial acid hydrolysis of barley husk hemicellulose.[3] Whistler and McGilvray[1] suggested that the positive rotation of the disaccharide was indicative of an α-D-xylopyranosyl linkage. Approximate calculations of the expected optical rotations of O-D-xylopyranosyl-L-arabopyranoses may be made using Hudson's rule.[4] For example, the molecular rotation of O-β-D-xylopyranosyl-α-L-arabopyranose is given by the following expression, $[M]_D$ = B(D-xylose) - A(methyl D-xylopyranoside) + B(L-arabinose) + A(L-arabinose). The table shows the calculated optical

rotations together with the observed physical constants for the known O-D-xylopyranosyl-L-arabinoses. Although the calculations do not take into account the relatively small differences likely to arise from linkages through different possible positions in the arabinose residue, the observed rotation for the 2-linked disaccharide is clearly consistent only with the presence of a β-D-xylopyranosyl linkage. Furthermore, the high optical rotation of 3-O-α-D-xylopyranosyl-L-arabinose, isolated from the partial acid hydrolysis of golden apple gum, [5] and of corn fibre hemicellulose, [6] indicates that the two disaccharides probably have different configurations at the glycosidic linkage. We now report confirmation of the structure of the disaccharide by a synthesis of 2-O-β-D-xylopyranosyl-L-arabinose. Since the appearance of a preliminary account [7] of some of the following results, Charlson, Gorin, and Perlin [8] have proved the presence of a β-glycosidic link in the disaccharide by its degradation to 2-O-β-D-xylopyranosylglycerol.

Disaccharide	M.p.	$[\alpha]_D$	Source
2-O-β-D-Xylopyranosyl-L-arabinose (anhydrous form)	167-168°	+32.9°	Corn cob hemicellulose B
monohydrate	80-81°	+47.0°→+32.5°	
trihydrate	97-99°	+53.7°→+33.2°	Barley husk hemicellulose
	96-98°	+55° ⟶ +31.5°	Synthesis
	98-99°	+49° ⟶ +30°	Esparto hemicellulose
3-O-α-D-Xylopyranosyl-L-arabinose	123°	+173°	Golden apple gum
	117.5-119°	+166°⟶+181.8°	Corn fibre hemicellulose
O-α-D-Xylopyranosyl-α-L-arabopyranose	---	+131°(calc.)	---
O-α-D-Xylopyranosyl-β-L-arabopyranose	---	+191°(calc.)	---
O-β-D-Xylopyranosyl-α-L-arabopyranose	---	+2°(calc.)	---
O-β-D-Xylopyranosyl-β-L-arabopyranose	---	+63°(calc.)	---

Since O-acyl-α-D-glycosyl halides react with alcohols to give the corresponding β-glycosides[9] the required glycosidic bond of the disaccharide was formed by a Koenigs-Knorr condensation[10] of 2:3:4-tri-O-acetyl-α-D-xylopyranosyl bromide with benzyl 3:4-O-isopropylidene-β-L-arabopyranoside. The condensation product, benzyl 3:4-O-isopropylidene-2-O-(2:3:4-tri-O-acetyl-β-D-xylopyranosyl)-β-L-arabopyranoside (I), was isolated as a crystalline compound after chromatography on alumina. Deacetylation of compound (I) gave benzyl 3:4-O-isopropylidene-2-O-β-D-xylopyranosyl-β-L-arabopyranoside (II), which on hydrogenation over palladium-calcium carbonate afforded 3:4-O-isopropylidene-2-β-D-xylopyranosyl-L-arabopyranose monohydrate (III). Mild acid hydrolysis of the isopropylidene compound (III) furnished the required disaccharide, which was readily separated from small amounts of xylose and arabinose and crystallised as 2-O-β-D-xylopyranosyl-L-arabinose trihydrate, identical (m.p. and mixed m.p., optical rotation, and X-ray single crystal photograph) with the disaccharide previously isolated from the partial acid hydrolysis of barley husk hemicellulose.[3]

A second crystalline condensation product (A) was isolated in low yield from the Koenigs-Knorr reaction. It is probable that this compound is a partially acetylated benzyl di-O-(xylosyl)arabinose since hydrolysis gave xylose and arabinose in

the proportion of 1.8 to 1, and since the infra-red spectrum contained a sharp absorption at 3531 cm.$^{-1}$ showing the presence of a single hydroxyl group in the molecule. The product (B) of deacetylation consumed 4.4 mol. of periodate, slightly more than the expected 4 mol. Methylation and deacetylation of A gave a methylated trisaccharide, hydrolysis of which gave 2:3:4-tri-O-methylxylose and a mono-O-methylarabinose. The latter sugar was shown to be the 2-methyl ether since chromatography of the periodate oxidation products[11] showed methoxymalondialdehyde and since the derived mixture of methyl pyranosides was attacked by periodate. Although insufficient of the trisaccharide derivative (A) was available for a complete characterisation, these experiments suggest that B is benzyl 3:4-di-O-(β-D-xylopyranosyl)-β-L-arabopyranoside and that A is a partially acetylated derivative. Although there is no apparent reason for the instability of benzyl 3:4-O-isopropylidene-β-L-arabopyranoside during the Koenigs-Knorr reaction, it is clear that some hydrolysis of the isopropylidene group took place since benzyl β-L-arabopyranoside was also isolated from the reaction mixture. The trisaccharide derivative (A) must have been formed by condensation of tri-O-acetyl-D-xylopyranosyl bromide with benzyl β-L-arabopyranoside.

In the course of the isolation of xylobiose and xylotriose from the partial acid hydrolysis of esparto hemicellulose,[12]

a small fraction was obtained which contained xylobiose and a second disaccharide having the same chromatographic mobility as 2-O-β-D-xylopyranosyl-L-arabinose.* This compound was separated chromatographically and was isolated as the crystalline 2-O-β-D-xylopyranosyl-L-arabinose trihydrate. It is clear that this disaccharide arises from a hitherto unrecognised structural feature of esparto hemicellulose. Previous investigations have shown that esparto hemicellulose contains at least two xylans, one devoid of arabinose residues,[13] and one containing arabinose residues,[14] the majority of which are present as end groups in the furanose form. The structural significance of the isolation of 2-O-β-D-xylopyranosyl-L-arabinose from esparto hemicellulose is not yet clear. It may be recalled, however, that a small quantity of an unidentified di-O-methylarabinose was isolated from the hydrolysis of methylated esparto araboxylan;[14] the optical rotation and chromatographic mobility of this sugar were similar to those of 3:5-di-O-methyl-L-arabinose. 1:2-Linked L-arabofuranose residues would be present in esparto hemicellulose if 2-O-β-D-xylopyranosyl-L-arabofuranosyl side-chains were attached to the backbone of 1:4-linked β-D-xylopyranose residues as in barley husk hemicellulose.[3]

* We are grateful to Dr. Mary E. Carter for drawing our attention to the presence of this disaccharide.

EXPERIMENTAL

Alumina, Type H, 100/200 S mesh, supplied by Peter Spence and Sons, Ltd., was shaken with N-acetic acid, washed with water by decantation until free from acid, and dried at 260°. Optical rotations were observed at 18° ± 2°.

Benzyl 3:4-O-isopropylidene-β-L-arabopyranoside.- Benzyl β-L-arabopyranoside (20 g.) in acetone (1 l.) was shaken with cupric sulphate (60 g.) and concentrated sulphuric acid (1 ml.) for 24 hr. The acetone solution was separated, acid was neutralised by passing in dry ammonia, salts were removed by filtration, and the solution was concentrated to a syrup. The syrup was extracted with ether and the extract (23 g.) was redissolved in ether and chromatographed on alumina (25 x 5 cm.). Elution with ether containing 5% ethanol, followed by two recrystallisations from light petroleum (b.p. 60-80°)-ether (3 : 1) afforded benzyl 3:4-O-isopropylidene-β-L-arabopyranoside (10 g.), m.p. 57-58°, $[\alpha]_D$ +187° (c 0.9 in $CHCl_3$) (Found: C, 64.7; H, 7.2. $C_{15}H_{20}O_5$ requires C, 64.3; H, 7.2%). Ballou[15] reports m.p. 55-58° and $[\alpha]_D$ -208° (EtOH) for benzyl 3:4-O-isopropylidene-β-D-arabopyranoside.

Condensation of 2:3:4-Tri-O-acetyl-α-D-xylopyranosyl Bromide and Benzyl 3:4-O-isopropylidene-β-L-arabopyranoside.- Benzyl 3:4-O-isopropylidene-β-L-arabopyranoside (9.2 g.), freshly prepared silver carbonate (15 g.), and anhydrous calcium sulphate

(40 g.) were shaken overnight in benzene (100 ml.). After addition of iodine (3 g.), a solution of 2:3:4-tri-O-acetyl-α-D-xylopyranosyl bromide (11.1 g.) in benzene (100 ml.) was added slowly with stirring during 1 hr. The mixture was shaken in the dark for 3 days (with occasional release of carbon dioxide) until the benzene solution gave no opalescence with ethanolic silver nitrate. Filtration of the reaction mixture followed by concentration of the filtrate yielded a syrup (16 g.), which was dissolved in benzene and chromatographed on alumina (25 x 5 cm.) to give six fractions.

Fraction 1. The crystalline solid (1.8 g., eluted with light petroleum-benzene (1 : 1)) after two recrystallisations from light petroleum containing a little ethanol gave benzyl 3:4-O-isopropylidene-2-O-(2:3:4-tri-O-acetyl-β-D-xylopyranosyl)-β-L-arabopyranoside (I) (1.35 g.), m.p. 110-111°, $[\alpha]_D$ +71° (c 1.0 in $CHCl_3$) and $[\alpha]_D$ +78° (c 1.0 in MeOH) (Found: C, 58.3; H, 6.4. $C_{26}H_{34}O_{12}$ requires C, 58.0; H, 6.4%).

Fraction 2. The syrup (0.6 g., eluted with light petroleum-benzene (1 : 3)) was dissolved in ethanol, crystalline material was deposited, and recrystallisation from ethanol gave compound A (200 mg.), m.p. 154-155°, $[\alpha]_D$ +38° (c 1.0 in $CHCl_3$). Hydrolysis of a sample of A gave xylose and arabinose in the ratio of 1.8: 1 (quantitative chromatography[16]). Deacetylation of A with sodium methoxide gave compound B, which after

recrystallisation from ethanol-water had m.p. 261-263° (Found: C, 52.0; H, 6.3. Benzyl di-O-(β-D-xylopyranosyl)-β-L-arabopyranoside ($C_{22}H_{32}O_{13}$) requires C, 42.4; H, 6.3%). The benzyl glycoside B consumed 4.4 mol. of periodate (spectrophotometric determination[17]). Direct methylation of A with methyl sulphate and sodium hydroxide and hydrolysis of the methylated trisaccharide gave two components a and b, R_G 0.95 (cf. 2:3:4-tri-O-methyl-D-xylose) and 0.37 in butan-1-ol-ethanol-water (4 : 1 : 5, upper layer). Chromatography[11] of the periodate oxidation product of b showed methoxymalondialdehyde. Demethylation of b with hydrobromic acid gave arabinose. The derived mixture of syrupy methyl pyranosides consumed ca. ~~1 mol.~~ 1 mol. of periodate.

Fractions 3 and 4. Benzyl β-L-arabopyranoside, m.p. and mixed m.p. 172-173°, was deposited from ethanolic solutions of fractions 3 (2.1 g., eluted with benzene) and 4 (1.6 g., eluted with benzene-ether (1 : 1)).

Fraction 5. The syrup (2.1 g., eluted with ether-ethanol (1 : 1)) was dissolved in ethanol and a crystalline substance was deposited, m.p. 141-142° and $[\alpha]_D$ +69° (c 1.3 in $CHCl_3$) [cf. 2:3:4-tri-O-acetyl-D-xylose, m.p. 138-141° and $[\alpha]_D$ +70° ($CHCl_3$)[18]]

Fraction 6. The mobile oil (3.6 g., eluted with acetone), $[\alpha]_D$ +10° (c 1.2 in H_2O), when hydrolysed, gave approximately equal amounts of xylose and arabinose.

Benzyl 3:4-O-isopropylidene-2-O-(β-D-xylopyranosyl)-β-L-arabopyranoside.(II).- Methanolic 0.1N-sodium methoxide (6 ml.) was added to the condensation product (I) (1.20 g.) in methanol (60 ml.), and after shaking the mixture for 6 hr. a crystalline substance separated. Recrystallisation from methanol afforded benzyl 3:4-O-isopropylidene-2-O-(β-D-xylopyranosyl)-β-L-arabopyranoside (II) (0.75 g.), m.p. 216-217°, $[\alpha]_D$ +132° (c 0.5 in MeOH) (Found: C, 58.2; H, 6.6. $C_{20}H_{28}O_9$ requires C, 58.3; H, 6.8%).

2-O-β-D-Xylopyranosyl-L-arabinose.- The benzyl glycoside (II) (0.72 g.) in ethanol-water (100 ml.) was shaken in hydrogen at atmospheric pressure in the presence of palladium hydroxide (5%)-calcium carbonate (4 g.) for 6 hr. Catalyst was removed by filtration, and the filtrate was concentrated to a syrup (0.55 g.). Extraction of the product with boiling acetone, followed by removal of solvent, afforded a crystalline compound (III) (0.50 g.), m.p. 141-143°, $[\alpha]_D$ +53° (equil.) (c 0.7 in H_2O). Although no satisfactory solvent could be found for recrystallisation, this substance was probably 3:4-O-isopropylidene-2-O-(β-D-xylopyranosyl)-L-arabinose monohydrate (Found: C, 45.3; H, 7.0. $C_{13}H_{22}O_9.H_2O$ requires C, 45.9; H, 7.1%). This substance (0.28 g.) was heated at 100° for 40 min. with 0.005N-oxalic acid (10 ml.), the solution was neutralised with Amberlite resin IR-4B(OH), and the filtrate

was concentrated to a syrup (0.20 g.). Recrystallisation from ethanol-water furnished 2-O-β-D-xylopyranosyl-L-arabinose trihydrate (120 mg.), which had m.p. and mixed m.p. 96-98°, and $[\alpha]_D$ +55° (3 min.) ⟶ +31.5° (90 min., const.) (c 1.5 in H_2O), and gave an X-ray single crystal photograph identical with that of an authentic sample.

Isolation of 2-O-β-D-Xylopyranosyl-L-arabinose from the Partial Hydrolysis of Esparto Hemicellulose.- During the isolation of xylobiose and xylotriose from the partial acid hydrolysis of esparto hemicellulose,[12] a small fraction (350 mg.) was obtained from the fractionation on a charcoal-celite column which was shown by paper chromatography in ethyl acetate-pyridine-water (10 : 4 : 3) to contain two components, xylobiose and an unidentified sugar, having R_{xylose} 0.59 and 0.67. The sugars were separated chromatographically on filter sheets and the faster-moving component (50 mg.) crystallised from ethanol-water to give 2-O-β-D-xylopyranosyl-L-arabinose trihydrate (20 mg.), m.p. 98-99°, $[\alpha]_D$ +49° (3 min.) ⟶ +30° (1 hr., const.) (c 1.0 in H_2O). The sugar was chromatographically indistinguishable in three solvent systems from the authentic disaccharide, hydrolysis gave xylose and arabinose, and hydrolysis of the derived aldobionic acid (bromine oxidation) gave only xylose. An X-ray single crystal photograph was identical with that of the disaccharide isolated from barley husk hemicellulose.[3]

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